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- (71) Applicant: ZYMOGENETICS, INC. [US/US]; 1201 Eastlake Avenue East, Seattle, WA 98102 (US).
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- (72) Inventors: KUESTNER, Rolf, E.; 24208 23rd Avenue West, Bothell, WA 98021 (US). GRANT, Francis, J.; 7714 37th NE, Seattle, WA 98115 (US). PRESNELL, Scott, R.; 2902 N. Puget Sound Avenue, Tacoma, WA 98407 (US).
- (74) Agent: WALSH, Brian, J.; ZymoGenetics, Inc., 1201 Eastlake Avenue East, Seattle, WA 98102 (US).
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(54) Title: HUMAN CYTOKINE RECEPTOR

(57) Abstract: Cytokines and their receptors have proven usefulness in both basic research and as therapeutics. The present invention provides a new human cytokine receptor designated as "Zcytor21".

## HUMAN CYTOKINE RECEPTOR

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## TECHNICAL FIELD

The present invention relates generally to a new protein expressed by human cells. In particular, the present invention relates to a novel gene that encodes a  
10 receptor, designated as "Zcytor21," and to nucleic acid molecules encoding Zcytor21 polypeptides.

## BACKGROUND OF THE INVENTION

Cytokines are soluble, small proteins that mediate a variety of biological  
15 effects, including the regulation of the growth and differentiation of many cell types (see, for example, Arai *et al.*, *Annu. Rev. Biochem.* 59:783 (1990); Mosmann, *Curr. Opin. Immunol.* 3:311 (1991); Paul and Seder, *Cell* 76:241 (1994)). Proteins that constitute the cytokine group include interleukins, interferons, colony stimulating factors, tumor necrosis factors, and other regulatory molecules. For example, human interleukin-17 is a  
20 cytokine which stimulates the expression of interleukin-6, intracellular adhesion molecule 1, interleukin-8, granulocyte macrophage colony-stimulating factor, and prostaglandin E2 expression, and plays a role in the preferential maturation of CD34+ hematopoietic precursors into neutrophils (Yao *et al.*, *J. Immunol.* 155:5483 (1995); Fossiez *et al.*, *J. Exp. Med.* 183:2593 (1996)).

25 Receptors that bind cytokines are typically composed of one or more integral membrane proteins that bind the cytokine with high affinity and transduce this binding event to the cell through the cytoplasmic portions of the certain receptor subunits. Cytokine receptors have been grouped into several classes on the basis of similarities in their extracellular ligand binding domains. For example, the receptor  
30 chains responsible for binding and/or transducing the effect of interferons are members of the type II cytokine receptor family, based upon a characteristic 200 residue extracellular domain.

The demonstrated *in vivo* activities of cytokines and their receptors illustrate the clinical potential of, and need for, other cytokines, cytokine receptors, cytokine agonists, and cytokine antagonists.

## 5 SUMMARY OF THE INVENTION

The present invention provides isolated polypeptides comprising an amino acid sequence that is at least 70%, at least 80%, or at least 90% identical to a reference amino acid sequence selected from the group consisting of: (a) amino acid residues 24 to 667 of SEQ ID NO:11, (b) amino acid residues 24 to 589 of SEQ ID  
10 NO:2, (c) amino acid residues 24 to 609 of SEQ ID NO:5, (d) amino acid residues 24 to 533 of SEQ ID NO:8, (e) amino acid residues 24 to 657 of SEQ ID NO:15, (f) amino acid residues 24 to 454 of SEQ ID NO:11, (g) amino acid residues 24 to 376 of SEQ ID NO:2, (h) amino acid residues 24 to 396 of SEQ ID NO:5, or (i) amino acid residues 24 to 444 of SEQ ID NO:15. Certain of these polypeptides can specifically bind with an  
15 antibody that specifically binds with a polypeptide consisting of the amino acid sequence of SEQ ID NOs:2, 5, 8, 11, or 15. Illustrative polypeptides include polypeptides comprising, or consisting of, amino acid residues 24 to 454 of SEQ ID NO:11, amino acid residues 24 to 376 of SEQ ID NO:2, amino acid residues 24 to 396 of SEQ ID NO:5, amino acid residues 24 to 444 of SEQ ID NO:15, or amino acid residues 24 to 533  
20 of SEQ ID NO:8.

The present invention also provides isolated polypeptides comprising at least 15 contiguous amino acid residues of an amino acid sequence selected from the group consisting of: (a) amino acid residues 24 to 667 of SEQ ID NO:11, (b) amino acid residues 24 to 589 of SEQ ID NO:2, (c) amino acid residues 24 to 609 of SEQ ID NO:5,  
25 (d) amino acid residues 24 to 533 of SEQ ID NO:8, (e) amino acid residues 24 to 657 of SEQ ID NO:15, (f) amino acid residues 24 to 454 of SEQ ID NO:11, (g) amino acid residues 24 to 376 of SEQ ID NO:2, (h) amino acid residues 24 to 396 of SEQ ID NO:5, or (i) amino acid residues 24 to 444 of SEQ ID NO:15. The present invention further provides isolated polypeptides comprising at least 30 contiguous amino acid residues of  
30 an amino acid sequence selected from the group consisting of: (a) amino acid residues 24 to 667 of SEQ ID NO:11, (b) amino acid residues 24 to 589 of SEQ ID NO:2, (c) amino

acid residues 24 to 609 of SEQ ID NO:5, (d) amino acid residues 24 to 533 of SEQ ID NO:8, (e) amino acid residues 24 to 657 of SEQ ID NO:15, (f) amino acid residues 24 to 454 of SEQ ID NO:11, (g) amino acid residues 24 to 376 of SEQ ID NO:2, (h) amino acid residues 24 to 396 of SEQ ID NO:5, or (i) amino acid residues 24 to 444 of SEQ ID NO:15. Illustrative polypeptides include polypeptides that either comprise, or consist of,  
5 amino acid residues (a) to (i).

The present invention also includes variant Zcytor21 polypeptides, wherein the amino acid sequence of the variant polypeptide shares a sequence identity with amino acid residues 24 to 454 of SEQ ID NO:11, amino acid residues 24 to 376 of  
10 SEQ ID NO:2, amino acid residues 24 to 396 of SEQ ID NO:5, amino acid residues 24 to 533 of SEQ ID NO:8, or amino acid residues 24 to 444, and wherein any difference between the amino acid sequence of the variant polypeptide and the corresponding amino acid sequence of SEQ ID NOs:2, 5, 8, 11, or 15, may be due to one or more conservative amino acid substitutions. The sequence identity may be at least 70%  
15 sequence identity, at least 80% sequence identity, at least sequence 90% identity, at least 95% sequence identity, or greater than 95% sequence identity.

The present invention further provides antibodies and antibody fragments that specifically bind with such polypeptides. Exemplary antibodies include polyclonal antibodies, murine monoclonal antibodies, humanized antibodies derived from murine  
20 monoclonal antibodies, and human monoclonal antibodies. Illustrative antibody fragments include F(ab')<sub>2</sub>, F(ab)<sub>2</sub>, Fab', Fab, Fv, scFv, and minimal recognition units. The present invention further includes compositions comprising a carrier and a peptide, polypeptide, or antibody described herein.

The present invention also provides isolated nucleic acid molecules that  
25 encode a Zcytor21 polypeptide, wherein the nucleic acid molecule is selected from the group consisting of: (a) a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NOs:3, 6, 9, 12, or 16, (b) a nucleic acid molecule encoding an amino acid sequence that comprises amino acid residues 24 to 454 of SEQ ID NO:11, amino acid residues 24 to 376 of SEQ ID NO:2, amino acid residues 24 to 396 of SEQ ID NO:5,  
30 amino acid residues 24 to 533 of SEQ ID NO:8, or amino acid residues 24 to 444 of SEQ ID NO:15, and (c) a nucleic acid molecule that remains hybridized following stringent



wash conditions to a nucleic acid molecule comprising the nucleotide sequence of nucleotides 66 to 2066 of SEQ ID NO:10, the nucleotide sequence of nucleotides 135 to 1427 of SEQ ID NO:10, the complement of the nucleotide sequence of nucleotides 66 to 2066 of SEQ ID NO:10, or the complement of the nucleotide sequence of nucleotides 135 to 1427 of SEQ ID NO:10. Illustrative nucleic acid molecules include those in which any difference between the amino acid sequence encoded by nucleic acid molecule (c) and the corresponding amino acid sequence of SEQ ID NO:11 is due to a conservative amino acid substitution. Similarly, nucleic acid molecules used for hybridization can be derived from the nucleotide sequences of SEQ ID NOs: 1, 4, 7, or 14.

The present invention also includes vectors and expression vectors comprising such nucleic acid molecules. Such expression vectors may comprise a transcription promoter, and a transcription terminator, wherein the promoter is operably linked with the nucleic acid molecule, and wherein the nucleic acid molecule is operably linked with the transcription terminator. The present invention further includes recombinant host cells and recombinant viruses comprising these vectors and expression vectors. Illustrative host cells include avian, bacterial, yeast, fungal, insect, mammalian, and plant cells. Recombinant host cells comprising such expression vectors can be used to produce Zcytor21 polypeptides by culturing such recombinant host cells that comprise the expression vector and that produce the Zcytor21 protein, and, optionally, isolating the Zcytor21 protein from the cultured recombinant host cells. The present invention also provides polypeptide products produced by such methods.

In addition, the present invention provides pharmaceutical compositions comprising a pharmaceutically acceptable carrier and at least one of such an expression vector or recombinant virus comprising such expression vectors. The present invention further includes pharmaceutical compositions, comprising a pharmaceutically acceptable carrier and a polypeptide described herein.

The present invention also contemplates methods for detecting the presence of Zcytor21 RNA in a biological sample, comprising the steps of (a) contacting a Zcytor21 nucleic acid probe under hybridizing conditions with either (i) test RNA molecules isolated from the biological sample, or (ii) nucleic acid molecules synthesized

from the isolated RNA molecules, wherein the probe has a nucleotide sequence comprising a portion of the nucleotide sequence of SEQ ID NO:1, or its complement, and (b) detecting the formation of hybrids of the nucleic acid probe and either the test RNA molecules or the synthesized nucleic acid molecules following stringent wash  
5 conditions, wherein the presence of the hybrids indicates the presence of *Zcytor21* RNA in the biological sample. For example, suitable probes can consist of the following nucleotide sequences: nucleic acid molecules consisting of the nucleotide sequence of SEQ ID NO:10, nucleic acid molecules consisting of the nucleotide sequence of nucleotides 66 to 2066 of SEQ ID NO:10, nucleotide sequence of nucleotides 135 to  
10 1427 of SEQ ID NO:10, and the like. Other suitable probes consist of the complement of these nucleotide sequences, or a portion of the nucleotide sequences or their complements.

The present invention further provides methods for detecting the presence of *Zcytor21* polypeptide in a biological sample, comprising the steps of: (a) contacting  
15 the biological sample with an antibody or an antibody fragment that specifically binds with a polypeptide comprising, or consisting of, the amino acid sequence of SEQ ID NOs:2, 5, 8, 11, or 15 wherein the contacting is performed under conditions that allow the binding of the antibody or antibody fragment to the biological sample, and (b) detecting any of the bound antibody or bound antibody fragment. Such an antibody or  
20 antibody fragment may further comprise a detectable label selected from the group consisting of radioisotope, fluorescent label, chemiluminescent label, enzyme label, bioluminescent label, and colloidal gold.

The present invention also provides kits for performing these detection methods. For example, a kit for detection of *Zcytor21* gene expression may comprise a  
25 container that comprises a nucleic acid molecule, wherein the nucleic acid molecule is selected from the group consisting of (a) a nucleic acid molecule comprising the nucleotide sequence of nucleotides 66 to 2066 of SEQ ID NO:10, (b) a nucleic acid molecule comprising the complement of nucleotides 66 to 2066 of the nucleotide sequence of SEQ ID NO:10, (c) a nucleic acid molecule comprising the nucleotide  
30 sequence of nucleotides 135 to 1427 of SEQ ID NO:10, (d) a nucleic acid molecule comprising the complement of nucleotides 135 to 1427 of the nucleotide sequence of

SEQ ID NO:10, and (e) a nucleic acid molecule that is a fragment of (a)-(d) consisting of at least eight nucleotides. Such a kit may also comprise a second container that comprises one or more reagents capable of indicating the presence of the nucleic acid molecule. On the other hand, a kit for detection of Zcytor21 protein may comprise a  
5 container that comprises an antibody, or an antibody fragment, that specifically binds with a polypeptide comprising, or consisting of, the amino acid sequence of SEQ ID NOs:2, 5, 8, 11, or 15.

The present invention also contemplates anti-idiotypic antibodies, or anti-idiotypic antibody fragments, that specifically bind an antibody or antibody fragment that  
10 specifically binds a polypeptide comprising, or consisting of, the amino acid sequence of SEQ ID NOs:2, 5, 8, 11, or 15. An exemplary anti-idiotypic antibody binds with an antibody that specifically binds a polypeptide consisting of amino acid residues 24 to 454 of SEQ ID NO:11, amino acid residues 24 to 376 of SEQ ID NO:2, amino acid residues 24 to 396 of SEQ ID NO:5, amino acid residues 24 to 533 of SEQ ID NO:8, or amino  
15 acid residues 24 to 444 of SEQ ID NO:15.

The present invention also provides isolated nucleic acid molecules comprising a nucleotide sequence that encodes a Zcytor21 secretion signal sequence and a nucleotide sequence that encodes a biologically active polypeptide, wherein the Zcytor21 secretion signal sequence comprises an amino acid sequence of residues 1 to 23  
20 of SEQ ID NO:2. Illustrative biologically active polypeptides include Factor VIIa, proinsulin, insulin, follicle stimulating hormone, tissue type plasminogen activator, tumor necrosis factor, interleukin, colony stimulating factor, interferon, erythropoietin, and thrombopoietin. Moreover, the present invention provides fusion proteins comprising a Zcytor21 secretion signal sequence and a polypeptide, wherein the  
25 Zcytor21 secretion signal sequence comprises an amino acid sequence of residues 1 to 23 of SEQ ID NO:2.

The present invention also provides fusion proteins, comprising a Zcytor21 polypeptide and an immunoglobulin moiety. Illustrative Zcytor21 polypeptides include polypeptides comprising amino acid residues 24 to 454 of SEQ ID NO:11, amino  
30 acid residues 24 to 376 of SEQ ID NO:2, amino acid residues 24 to 396 of SEQ ID NO:5, amino acid residues 24 to 533 of SEQ ID NO:8, or amino acid residues 24 to 444

of SEQ ID NO:15. In such fusion proteins, the immunoglobulin moiety may be an immunoglobulin heavy chain constant region, such as a human F<sub>C</sub> fragment. The present invention further includes isolated nucleic acid molecules that encode such fusion proteins.

5                    These and other aspects of the invention will become evident upon reference to the following detailed description.

## DETAILED DESCRIPTION OF THE INVENTION

### 1. Overview

10                    The present invention provides a novel receptor, designated "Zcytor21." The present invention also provides Zcytor21 polypeptides and Zcytor21 fusion proteins, as well as nucleic acid molecules encoding such polypeptides and proteins, and methods for using these nucleic acid molecules and amino acid sequences.

                    Four splice variants of Zcytor21 have been identified. The variants have  
15                    been designated as Zcytor21-d2, Zcytor21-f1, Zcytor21-f5, Zcytor21-f6, and Zcytor21-g13. Table 1 provides the corresponding sequence identification numbers for the nucleotide and amino acid sequences of the variants, while Table 2 shows structural features of the Zcytor21 polypeptides. The *Zcytor21* gene resides in human chromosome 3p25.3.

20

Table 1

Zcytor21 Type	Sequence Type		
	Nucleotide	Amino Acid	Degenerate
Zcytor21-f1	1	2	3
Zcytor21-f5	4	5	6
Zcytor21-f6	7	8	9
Zcytor21-d2	10	11	12
Zcytor21-g13	14	15	16

Table 2

Protein	Location of Structural Features (amino acid residues within sequence)			
	Signal Sequence	Extracellular Domain	Transmembrane Domain	Intracellular Domain
Zcytor21-d2 (SEQ ID NO:11)	1-23	24-454	455-477	478-667
Zcytor21-f1 (SEQ ID NO:2)	1-23	24-376	377-399	400-589
Zcytor21-f5 (SEQ ID NO:5)	1-23	24-396	397-419	420-609
Zcytor21-f6 (SEQ ID NO:8)	1-23	24-533		
Zcytor21-g13 (SEQ ID NO:15)	1-23	24-444	445-467	468-657

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The *Zcytor21* gene is expressed by human germ cell tumors and human skin cells. PCR analyses show that *Zcytor21* mRNA is present in human brain tissue, and tracheal tissue. In contrast, little or no evidence of gene expression was detected in adrenal gland, skeletal muscle, bladder, kidney, lung, and spleen. Thus, *Zcytor21* nucleotide and amino acid sequences can be used to differentiate tissues.

10

## 2. Definitions

In the description that follows, a number of terms are used extensively. The following definitions are provided to facilitate understanding of the invention.

15 Unless otherwise specified, "a," "an," "the," and "at least one" are used interchangeably and mean one or more than one.

As used herein, "nucleic acid" or "nucleic acid molecule" refers to polynucleotides, such as deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), oligonucleotides, fragments generated by the polymerase chain reaction (PCR), and

fragments generated by any of ligation, scission, endonuclease action, and exonuclease action. Nucleic acid molecules can be composed of monomers that are naturally-occurring nucleotides (such as DNA and RNA), or analogs of naturally-occurring nucleotides (*e.g.*,  $\alpha$ -enantiomeric forms of naturally-occurring nucleotides), or a  
5 combination of both. Modified nucleotides can have alterations in sugar moieties and/or in pyrimidine or purine base moieties. Sugar modifications include, for example, replacement of one or more hydroxyl groups with halogens, alkyl groups, amines, and azido groups, or sugars can be functionalized as ethers or esters. Moreover, the entire sugar moiety can be replaced with sterically and electronically similar structures, such as  
10 aza-sugars and carbocyclic sugar analogs. Examples of modifications in a base moiety include alkylated purines and pyrimidines, acylated purines or pyrimidines, or other well-known heterocyclic substitutes. Nucleic acid monomers can be linked by phosphodiester bonds or analogs of such linkages. Analogs of phosphodiester linkages include phosphorothioate, phosphorodithioate, phosphoroselenoate, phosphorodiselenoate,  
15 phosphoroanilothioate, phosphoranilidate, phosphoramidate, and the like. The term "nucleic acid molecule" also includes so-called "peptide nucleic acids," which comprise naturally-occurring or modified nucleic acid bases attached to a polyamide backbone. Nucleic acids can be either single stranded or double stranded.

The term "complement of a nucleic acid molecule" refers to a nucleic acid  
20 molecule having a complementary nucleotide sequence and reverse orientation as compared to a reference nucleotide sequence. For example, the sequence 5' ATGCACGGG 3' is complementary to 5' CCCGTGCAT 3'.

The term "contig" denotes a nucleic acid molecule that has a contiguous stretch of identical or complementary sequence to another nucleic acid molecule.  
25 Contiguous sequences are said to "overlap" a given stretch of a nucleic acid molecule either in their entirety or along a partial stretch of the nucleic acid molecule.

The term "degenerate nucleotide sequence" denotes a sequence of nucleotides that includes one or more degenerate codons as compared to a reference nucleic acid molecule that encodes a polypeptide. Degenerate codons contain different  
30 triplets of nucleotides, but encode the same amino acid residue (*i.e.*, GAU and GAC triplets each encode Asp).

The term "structural gene" refers to a nucleic acid molecule that is transcribed into messenger RNA (mRNA), which is then translated into a sequence of amino acids characteristic of a specific polypeptide.

An "isolated nucleic acid molecule" is a nucleic acid molecule that is not integrated in the genomic DNA of an organism. For example, a DNA molecule that encodes a growth factor that has been separated from the genomic DNA of a cell is an isolated DNA molecule. Another example of an isolated nucleic acid molecule is a chemically-synthesized nucleic acid molecule that is not integrated in the genome of an organism. A nucleic acid molecule that has been isolated from a particular species is smaller than the complete DNA molecule of a chromosome from that species.

A "nucleic acid molecule construct" is a nucleic acid molecule, either single- or double-stranded, that has been modified through human intervention to contain segments of nucleic acid combined and juxtaposed in an arrangement not existing in nature.

"Linear DNA" denotes non-circular DNA molecules having free 5' and 3' ends. Linear DNA can be prepared from closed circular DNA molecules, such as plasmids, by enzymatic digestion or physical disruption.

"Complementary DNA (cDNA)" is a single-stranded DNA molecule that is formed from an mRNA template by the enzyme reverse transcriptase. Typically, a primer complementary to portions of mRNA is employed for the initiation of reverse transcription. Those skilled in the art also use the term "cDNA" to refer to a double-stranded DNA molecule consisting of such a single-stranded DNA molecule and its complementary DNA strand. The term "cDNA" also refers to a clone of a cDNA molecule synthesized from an RNA template.

A "promoter" is a nucleotide sequence that directs the transcription of a structural gene. Typically, a promoter is located in the 5' non-coding region of a gene, proximal to the transcriptional start site of a structural gene. Sequence elements within promoters that function in the initiation of transcription are often characterized by consensus nucleotide sequences. These promoter elements include RNA polymerase binding sites, TATA sequences, CAAT sequences, differentiation-specific elements (DSEs; McGehee *et al.*, *Mol. Endocrinol.* 7:551 (1993)), cyclic AMP response elements

(CREs), serum response elements (SREs; Treisman, *Seminars in Cancer Biol.* 1:47 (1990)), glucocorticoid response elements (GREs), and binding sites for other transcription factors, such as CRE/ATF (O'Reilly *et al.*, *J. Biol. Chem.* 267:19938 (1992)), AP2 (Ye *et al.*, *J. Biol. Chem.* 269:25728 (1994)), SP1, cAMP response element  
5 binding protein (CREB; Loeken, *Gene Expr.* 3:253 (1993)) and octamer factors (see, in general, Watson *et al.*, eds., *Molecular Biology of the Gene*, 4th ed. (The Benjamin/Cummings Publishing Company, Inc. 1987), and Lemaigre and Rousseau, *Biochem. J.* 303:1 (1994)). If a promoter is an inducible promoter, then the rate of transcription increases in response to an inducing agent. In contrast, the rate of  
10 transcription is not regulated by an inducing agent if the promoter is a constitutive promoter. Repressible promoters are also known.

A "core promoter" contains essential nucleotide sequences for promoter function, including the TATA box and start of transcription. By this definition, a core promoter may or may not have detectable activity in the absence of specific sequences  
15 that may enhance the activity or confer tissue specific activity.

A "regulatory element" is a nucleotide sequence that modulates the activity of a core promoter. For example, a regulatory element may contain a nucleotide sequence that binds with cellular factors enabling transcription exclusively or preferentially in particular cells, tissues, or organelles. These types of regulatory  
20 elements are normally associated with genes that are expressed in a "cell-specific," "tissue-specific," or "organelle-specific" manner.

An "enhancer" is a type of regulatory element that can increase the efficiency of transcription, regardless of the distance or orientation of the enhancer relative to the start site of transcription.

25 "Heterologous DNA" refers to a DNA molecule, or a population of DNA molecules, that does not exist naturally within a given host cell. DNA molecules heterologous to a particular host cell may contain DNA derived from the host cell species (*i.e.*, endogenous DNA) so long as that host DNA is combined with non-host DNA (*i.e.*, exogenous DNA). For example, a DNA molecule containing a non-host  
30 DNA segment encoding a polypeptide operably linked to a host DNA segment comprising a transcription promoter is considered to be a heterologous DNA molecule.



Conversely, a heterologous DNA molecule can comprise an endogenous gene operably linked with an exogenous promoter. As another illustration, a DNA molecule comprising a gene derived from a wild-type cell is considered to be heterologous DNA if that DNA molecule is introduced into a mutant cell that lacks the wild-type gene.

5           A "polypeptide" is a polymer of amino acid residues joined by peptide bonds, whether produced naturally or synthetically. Polypeptides of less than about 10 amino acid residues are commonly referred to as "peptides."

          A "protein" is a macromolecule comprising one or more polypeptide chains. A protein may also comprise non-peptidic components, such as carbohydrate  
10   groups. Carbohydrates and other non-peptidic substituents may be added to a protein by the cell in which the protein is produced, and will vary with the type of cell. Proteins are defined herein in terms of their amino acid backbone structures; substituents such as carbohydrate groups are generally not specified, but may be present nonetheless.

          A peptide or polypeptide encoded by a non-host DNA molecule is a  
15   "heterologous" peptide or polypeptide.

          An "integrated genetic element" is a segment of DNA that has been incorporated into a chromosome of a host cell after that element is introduced into the cell through human manipulation. Within the present invention, integrated genetic elements are most commonly derived from linearized plasmids that are introduced into  
20   the cells by electroporation or other techniques. Integrated genetic elements are passed from the original host cell to its progeny.

          A "cloning vector" is a nucleic acid molecule, such as a plasmid, cosmid, or bacteriophage, which has the capability of replicating autonomously in a host cell. Cloning vectors typically contain one or a small number of restriction endonuclease recognition  
25   sites that allow insertion of a nucleic acid molecule in a determinable fashion without loss of an essential biological function of the vector, as well as nucleotide sequences encoding a marker gene that is suitable for use in the identification and selection of cells transformed with the cloning vector. Marker genes typically include genes that provide tetracycline resistance or ampicillin resistance.

30           An "expression vector" is a nucleic acid molecule encoding a gene that is expressed in a host cell. Typically, an expression vector comprises a transcription

promoter, a gene, and a transcription terminator. Gene expression is usually placed under the control of a promoter, and such a gene is said to be "operably linked to" the promoter. Similarly, a regulatory element and a core promoter are operably linked if the regulatory element modulates the activity of the core promoter.

5           A "recombinant host" is a cell that contains a heterologous nucleic acid molecule, such as a cloning vector or expression vector. In the present context, an example of a recombinant host is a cell that produces Zcytor21 from an expression vector. In contrast, Zcytor21 can be produced by a cell that is a "natural source" of Zcytor21, and that lacks an expression vector.

10           "Integrative transformants" are recombinant host cells, in which heterologous DNA has become integrated into the genomic DNA of the cells.

          A "fusion protein" is a hybrid protein expressed by a nucleic acid molecule comprising nucleotide sequences of at least two genes. For example, a fusion protein can comprise at least part of a Zcytor21 polypeptide fused with a polypeptide that  
15 binds an affinity matrix. Such a fusion protein provides a means to isolate large quantities of Zcytor21 using affinity chromatography.

          The term "receptor" denotes a cell-associated protein that binds to a bioactive molecule termed a "ligand." This interaction mediates the effect of the ligand on the cell. Receptors can be membrane bound, cytosolic or nuclear; monomeric (*e.g.*,  
20 thyroid stimulating hormone receptor, beta-adrenergic receptor) or multimeric (*e.g.*, PDGF receptor, growth hormone receptor, IL-3 receptor, GM-CSF receptor, G-CSF receptor, erythropoietin receptor and IL-6 receptor). Membrane-bound receptors are characterized by a multi-domain structure comprising an extracellular ligand-binding domain and an intracellular effector domain that is typically involved in signal  
25 transduction. In certain membrane-bound receptors, the extracellular ligand-binding domain and the intracellular effector domain are located in separate polypeptides that comprise the complete functional receptor.

          In general, the binding of ligand to receptor results in a conformational change in the receptor that causes an interaction between the effector domain and other  
30 molecule(s) in the cell, which in turn leads to an alteration in the metabolism of the cell. Metabolic events that are often linked to receptor-ligand interactions include gene

transcription, phosphorylation, dephosphorylation, increases in cyclic AMP production, mobilization of cellular calcium, mobilization of membrane lipids, cell adhesion, hydrolysis of inositol lipids and hydrolysis of phospholipids.

The term "secretory signal sequence" denotes a DNA sequence that  
5 encodes a peptide (a "secretory peptide") that, as a component of a larger polypeptide, directs the larger polypeptide through a secretory pathway of a cell in which it is synthesized. The larger polypeptide is commonly cleaved to remove the secretory peptide during transit through the secretory pathway.

An "isolated polypeptide" is a polypeptide that is essentially free from  
10 contaminating cellular components, such as carbohydrate, lipid, or other proteinaceous impurities associated with the polypeptide in nature. Typically, a preparation of isolated polypeptide contains the polypeptide in a highly purified form, *i.e.*, at least about 80% pure, at least about 90% pure, at least about 95% pure, greater than 95% pure, or greater than 99% pure. One way to show that a particular protein preparation contains an  
15 isolated polypeptide is by the appearance of a single band following sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis of the protein preparation and Coomassie Brilliant Blue staining of the gel. However, the term "isolated" does not exclude the presence of the same polypeptide in alternative physical forms, such as dimers or alternatively glycosylated or derivatized forms.

20 The terms "amino-terminal" and "carboxyl-terminal" are used herein to denote positions within polypeptides. Where the context allows, these terms are used with reference to a particular sequence or portion of a polypeptide to denote proximity or relative position. For example, a certain sequence positioned carboxyl-terminal to a reference sequence within a polypeptide is located proximal to the carboxyl terminus of  
25 the reference sequence, but is not necessarily at the carboxyl terminus of the complete polypeptide.

The term "expression" refers to the biosynthesis of a gene product. For example, in the case of a structural gene, expression involves transcription of the structural gene into mRNA and the translation of mRNA into one or more polypeptides.

30 The term "splice variant" is used herein to denote alternative forms of RNA transcribed from a gene. Splice variation arises naturally through use of alternative

splicing sites within a transcribed RNA molecule, or less commonly between separately transcribed RNA molecules, and may result in several mRNAs transcribed from the same gene. Splice variants may encode polypeptides having altered amino acid sequence. The term splice variant is also used herein to denote a polypeptide encoded by  
5 a splice variant of an mRNA transcribed from a gene.

As used herein, the term "immunomodulator" includes cytokines, stem cell growth factors, lymphotoxins, co-stimulatory molecules, hematopoietic factors, and synthetic analogs of these molecules.

The term "complement/anti-complement pair" denotes non-identical  
10 moieties that form a non-covalently associated, stable pair under appropriate conditions. For instance, biotin and avidin (or streptavidin) are prototypical members of a complement/anti-complement pair. Other exemplary complement/anti-complement pairs include receptor/ligand pairs, antibody/antigen (or hapten or epitope) pairs, sense/antisense polynucleotide pairs, and the like. Where subsequent dissociation of the  
15 complement/anti-complement pair is desirable, the complement/anti-complement pair preferably has a binding affinity of less than  $10^9 \text{ M}^{-1}$ .

An "anti-idiotypic antibody" is an antibody that binds with the variable region domain of an immunoglobulin. In the present context, an anti-idiotypic antibody binds with the variable region of an anti-Zcytor21 antibody, and thus, an anti-idiotypic  
20 antibody mimics an epitope of Zcytor21.

An "antibody fragment" is a portion of an antibody such as  $\text{F(ab')}_2$ ,  $\text{F(ab)}_2$ ,  $\text{Fab'}$ ,  $\text{Fab}$ , and the like. Regardless of structure, an antibody fragment binds with the same antigen that is recognized by the intact antibody. For example, an anti-Zcytor21 monoclonal antibody fragment binds with an epitope of Zcytor21.

25 The term "antibody fragment" also includes a synthetic or a genetically engineered polypeptide that binds to a specific antigen, such as polypeptides consisting of the light chain variable region, "Fv" fragments consisting of the variable regions of the heavy and light chains, recombinant single chain polypeptide molecules in which light and heavy variable regions are connected by a peptide linker ("scFv proteins"), and minimal  
30 recognition units consisting of the amino acid residues that mimic the hypervariable region.

A "chimeric antibody" is a recombinant protein that contains the variable domains and complementary determining regions derived from a rodent antibody, while the remainder of the antibody molecule is derived from a human antibody.

"Humanized antibodies" are recombinant proteins in which murine  
5 complementarity determining regions of a monoclonal antibody have been transferred from heavy and light variable chains of the murine immunoglobulin into a human variable domain.

As used herein, a "therapeutic agent" is a molecule or atom, which is conjugated to an antibody moiety to produce a conjugate, which is useful for therapy.  
10 Examples of therapeutic agents include drugs, toxins, immunomodulators, chelators, boron compounds, photoactive agents or dyes, and radioisotopes.

A "detectable label" is a molecule or atom, which can be conjugated to an antibody moiety to produce a molecule useful for diagnosis. Examples of detectable labels include chelators, photoactive agents, radioisotopes, fluorescent agents,  
15 paramagnetic ions, or other marker moieties.

The term "affinity tag" is used herein to denote a polypeptide segment that can be attached to a second polypeptide to provide for purification or detection of the second polypeptide or provide sites for attachment of the second polypeptide to a substrate. In principal, any peptide or protein for which an antibody or other specific  
20 binding agent is available can be used as an affinity tag. Affinity tags include a poly-histidine tract, protein A (Nilsson *et al.*, *EMBO J.* 4:1075 (1985); Nilsson *et al.*, *Methods Enzymol.* 198:3 (1991)), glutathione S transferase (Smith and Johnson, *Gene* 67:31 (1988)), Glu-Glu affinity tag (Grussenmeyer *et al.*, *Proc. Natl. Acad. Sci. USA* 82:7952 (1985)), substance P, FLAG peptide (Hopp *et al.*, *Biotechnology* 6:1204 (1988)),  
25 streptavidin binding peptide, or other antigenic epitope or binding domain. See, in general, Ford *et al.*, *Protein Expression and Purification* 2:95 (1991). DNA molecules encoding affinity tags are available from commercial suppliers (*e.g.*, Pharmacia Biotech, Piscataway, NJ).

A "naked antibody" is an entire antibody, as opposed to an antibody  
30 fragment, which is not conjugated with a therapeutic agent. Naked antibodies include

both polyclonal and monoclonal antibodies, as well as certain recombinant antibodies, such as chimeric and humanized antibodies.

As used herein, the term "antibody component" includes both an entire antibody and an antibody fragment.

5           An "immunoconjugate" is a conjugate of an antibody component with a therapeutic agent or a detectable label.

As used herein, the term "antibody fusion protein" refers to a recombinant molecule that comprises an antibody component and a Zcytor21 polypeptide component. Examples of an antibody fusion protein include a protein that comprises a Zcytor21  
10 extracellular domain, and either an Fc domain or an antigen-binding region.

A "target polypeptide" or a "target peptide" is an amino acid sequence that comprises at least one epitope, and that is expressed on a target cell, such as a tumor cell, or a cell that carries an infectious agent antigen. T cells recognize peptide epitopes presented by a major histocompatibility complex molecule to a target polypeptide or  
15 target peptide and typically lyse the target cell or recruit other immune cells to the site of the target cell, thereby killing the target cell.

An "antigenic peptide" is a peptide, which will bind a major histocompatibility complex molecule to form an MHC-peptide complex, which is recognized by a T cell, thereby inducing a cytotoxic lymphocyte response upon  
20 presentation to the T cell. Thus, antigenic peptides are capable of binding to an appropriate major histocompatibility complex molecule and inducing a cytotoxic T cells response, such as cell lysis or specific cytokine release against the target cell, which binds or expresses the antigen. The antigenic peptide can be bound in the context of a class I or class II major histocompatibility complex molecule, on an antigen presenting  
25 cell or on a target cell.

In eukaryotes, RNA polymerase II catalyzes the transcription of a structural gene to produce mRNA. A nucleic acid molecule can be designed to contain an RNA polymerase II template in which the RNA transcript has a sequence that is complementary to that of a specific mRNA. The RNA transcript is termed an "anti-  
30 sense RNA" and a nucleic acid molecule that encodes the anti-sense RNA is termed an

“anti-sense gene.” Anti-sense RNA molecules are capable of binding to mRNA molecules, resulting in an inhibition of mRNA translation.

An “anti-sense oligonucleotide specific for *Zcytor21*” or a “*Zcytor21* anti-sense oligonucleotide” is an oligonucleotide having a sequence (a) capable of forming a  
5 stable triplex with a portion of the *Zcytor21* gene, or (b) capable of forming a stable duplex with a portion of an mRNA transcript of the *Zcytor21* gene.

A “ribozyme” is a nucleic acid molecule that contains a catalytic center. The term includes RNA enzymes, self-splicing RNAs, self-cleaving RNAs, and nucleic acid molecules that perform these catalytic functions. A nucleic acid molecule that  
10 encodes a ribozyme is termed a “ribozyme gene.”

An “external guide sequence” is a nucleic acid molecule that directs the endogenous ribozyme, RNase P, to a particular species of intracellular mRNA, resulting in the cleavage of the mRNA by RNase P. A nucleic acid molecule that encodes an external guide sequence is termed an “external guide sequence gene.”

15 The term “variant *Zcytor21* gene” refers to nucleic acid molecules that encode a polypeptide having an amino acid sequence that is a modification of SEQ ID NO:11. Such variants include naturally-occurring polymorphisms of *Zcytor21* genes, as well as synthetic genes that contain conservative amino acid substitutions of the amino acid sequence of SEQ ID NO:11. Additional variant forms of *Zcytor21* genes are nucleic  
20 acid molecules that contain insertions or deletions of the nucleotide sequences described herein. A variant *Zcytor21* gene can be identified, for example, by determining whether the gene hybridizes with a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:10, or its complement, under stringent conditions.

Alternatively, variant *Zcytor21* genes can be identified by sequence  
25 comparison. Two amino acid sequences have “100% amino acid sequence identity” if the amino acid residues of the two amino acid sequences are the same when aligned for maximal correspondence. Similarly, two nucleotide sequences have “100% nucleotide sequence identity” if the nucleotide residues of the two nucleotide sequences are the same when aligned for maximal correspondence. Sequence comparisons can be  
30 performed using standard software programs such as those included in the LASERGENE bioinformatics computing suite, which is produced by DNASTAR (Madison,

Wisconsin). Other methods for comparing two nucleotide or amino acid sequences by determining optimal alignment are well-known to those of skill in the art (see, for example, Peruski and Peruski, *The Internet and the New Biology: Tools for Genomic and Molecular Research* (ASM Press, Inc. 1997), Wu *et al.* (eds.), "Information  
5 Superhighway and Computer Databases of Nucleic Acids and Proteins," in *Methods in Gene Biotechnology*, pages 123-151 (CRC Press, Inc. 1997), and Bishop (ed.), *Guide to Human Genome Computing*, 2nd Edition (Academic Press, Inc. 1998)). Particular methods for determining sequence identity are described below.

Regardless of the particular method used to identify a variant *Zcytor21*  
10 gene or variant *Zcytor21* polypeptide, a variant gene or polypeptide encoded by a variant gene may be functionally characterized the ability to bind specifically to an anti-*Zcytor21* antibody.

The term "allelic variant" is used herein to denote any of two or more alternative forms of a gene occupying the same chromosomal locus. Allelic variation  
15 arises naturally through mutation, and may result in phenotypic polymorphism within populations. Gene mutations can be silent (no change in the encoded polypeptide) or may encode polypeptides having altered amino acid sequence. The term allelic variant is also used herein to denote a protein encoded by an allelic variant of a gene.

The term "ortholog" denotes a polypeptide or protein obtained from one  
20 species that is the functional counterpart of a polypeptide or protein from a different species. Sequence differences among orthologs are the result of speciation.

"Paralogs" are distinct but structurally related proteins made by an organism. Paralogs are believed to arise through gene duplication. For example,  $\alpha$ -globin,  $\beta$ -globin, and myoglobin are paralogs of each other.

25 The present invention includes functional fragments of *Zcytor21* genes. Within the context of this invention, a "functional fragment" of a *Zcytor21* gene refers to a nucleic acid molecule that encodes a portion of a *Zcytor21* polypeptide, which is a domain described herein or at least specifically binds with an anti-*Zcytor21* antibody.

Due to the imprecision of standard analytical methods, molecular weights  
30 and lengths of polymers are understood to be approximate values. When such a value is



expressed as "about" X or "approximately" X, the stated value of X will be understood to be accurate to  $\pm 10\%$ .

### 3. *Production of Nucleic Acid Molecules That Encode Zcytor21*

5 Nucleic acid molecules encoding a human Zcytor21 can be obtained by screening a human cDNA or genomic library using polynucleotide probes based upon any of SEQ ID NOs:1, 4, 7, 10, 14. These techniques are standard and well-established.

As an illustration, a nucleic acid molecule that encodes human Zcytor21 can be isolated from a cDNA library. In this case, the first step would be to prepare the  
10 cDNA library by isolating RNA from a tissue, such as skin tissue, using methods well-known to those of skill in the art. In general, RNA isolation techniques must provide a method for breaking cells, a means of inhibiting RNase-directed degradation of RNA, and a method of separating RNA from DNA, protein, and polysaccharide contaminants. For example, total RNA can be isolated by freezing tissue in liquid nitrogen, grinding the  
15 frozen tissue with a mortar and pestle to lyse the cells, extracting the ground tissue with a solution of phenol/chloroform to remove proteins, and separating RNA from the remaining impurities by selective precipitation with lithium chloride (see, for example, Ausubel *et al.* (eds.), *Short Protocols in Molecular Biology*, 3<sup>rd</sup> Edition, pages 4-1 to 4-6 (John Wiley & Sons 1995) ["Ausubel (1995)"]; Wu *et al.*, *Methods in Gene Biotechnology*, pages 33-41  
20 (CRC Press, Inc. 1997) ["Wu (1997)"]).

Alternatively, total RNA can be isolated by extracting ground tissue with guanidinium isothiocyanate, extracting with organic solvents, and separating RNA from contaminants using differential centrifugation (see, for example, Chirgwin *et al.*, *Biochemistry* 18:52 (1979); Ausubel (1995) at pages 4-1 to 4-6; Wu (1997) at pages 33-  
25 41).

In order to construct a cDNA library, poly(A)<sup>+</sup> RNA must be isolated from a total RNA preparation. Poly(A)<sup>+</sup> RNA can be isolated from total RNA using the standard technique of oligo(dT)-cellulose chromatography (see, for example, Aviv and Leder, *Proc. Nat'l Acad. Sci. USA* 69:1408 (1972); Ausubel (1995) at pages 4-11 to 4-12).

30 Double-stranded cDNA molecules are synthesized from poly(A)<sup>+</sup> RNA using techniques well-known to those in the art. (see, for example, Wu (1997) at pages

41-46). Moreover, commercially available kits can be used to synthesize double-stranded cDNA molecules. For example, such kits are available from Life Technologies, Inc. (Gaithersburg, MD), CLONTECH Laboratories, Inc. (Palo Alto, CA), Promega Corporation (Madison, WI) and STRATAGENE (La Jolla, CA).

5                Various cloning vectors are appropriate for the construction of a cDNA library. For example, a cDNA library can be prepared in a vector derived from bacteriophage, such as a  $\lambda$ gt10 vector. See, for example, Huynh *et al.*, "Constructing and Screening cDNA Libraries in  $\lambda$ gt10 and  $\lambda$ gt11," in *DNA Cloning: A Practical Approach Vol. I*, Glover (ed.), page 49 (IRL Press, 1985); Wu (1997) at pages 47-52.

10              Alternatively, double-stranded cDNA molecules can be inserted into a plasmid vector, such as a PBLUESCRIPT vector (STRATAGENE; La Jolla, CA), a LAMDAGEM-4 (Promega Corp.) or other commercially available vectors. Suitable cloning vectors also can be obtained from the American Type Culture Collection (Manassas, VA).

15              To amplify the cloned cDNA molecules, the cDNA library is inserted into a prokaryotic host, using standard techniques. For example, a cDNA library can be introduced into competent *E. coli* DH5 cells, which can be obtained, for example, from Life Technologies, Inc. (Gaithersburg, MD).

                A human genomic library can be prepared by means well-known in the art  
20 (see, for example, Ausubel (1995) at pages 5-1 to 5-6; Wu (1997) at pages 307-327). Genomic DNA can be isolated by lysing tissue with the detergent Sarkosyl, digesting the lysate with proteinase K, clearing insoluble debris from the lysate by centrifugation, precipitating nucleic acid from the lysate using isopropanol, and purifying resuspended DNA on a cesium chloride density gradient.

25              DNA fragments that are suitable for the production of a genomic library can be obtained by the random shearing of genomic DNA or by the partial digestion of genomic DNA with restriction endonucleases. Genomic DNA fragments can be inserted into a vector, such as a bacteriophage or cosmid vector, in accordance with conventional techniques, such as the use of restriction enzyme digestion to provide appropriate termini,  
30 the use of alkaline phosphatase treatment to avoid undesirable joining of DNA molecules,

and ligation with appropriate ligases. Techniques for such manipulation are well-known in the art (see, for example, Ausubel (1995) at pages 5-1 to 5-6; Wu (1997) at pages 307-327).

Alternatively, human genomic libraries can be obtained from commercial sources such as Research Genetics (Huntsville, AL) and the American Type Culture  
5 Collection (Manassas, VA).

A library containing cDNA or genomic clones can be screened with one or more polynucleotide probes based upon SEQ ID NOs:1, 4, 7, 10, or 14, using standard methods (see, for example, Ausubel (1995) at pages 6-1 to 6-11).

Nucleic acid molecules that encode a human *Zcytor21* gene can also be  
10 obtained using the polymerase chain reaction (PCR) with oligonucleotide primers having nucleotide sequences that are based upon the nucleotide sequences of the *Zcytor21* gene, as described herein. General methods for screening libraries with PCR are provided by, for example, Yu *et al.*, "Use of the Polymerase Chain Reaction to Screen Phage Libraries," in *Methods in Molecular Biology, Vol. 15: PCR Protocols: Current Methods*  
15 *and Applications*, White (ed.), pages 211-215 (Humana Press, Inc. 1993). Moreover, techniques for using PCR to isolate related genes are described by, for example, Preston, "Use of Degenerate Oligonucleotide Primers and the Polymerase Chain Reaction to Clone Gene Family Members," in *Methods in Molecular Biology, Vol. 15: PCR Protocols: Current Methods and Applications*, White (ed.), pages 317-337 (Humana  
20 Press, Inc. 1993).

Anti-*Zcytor21* antibodies, produced as described below, can also be used to isolate DNA sequences that encode human *Zcytor21* genes from cDNA libraries. For example, the antibodies can be used to screen  $\lambda$ gt11 expression libraries, or the antibodies can be used for immunoscreening following hybrid selection and translation  
25 (see, for example, Ausubel (1995) at pages 6-12 to 6-16; Margolis *et al.*, "Screening  $\lambda$  expression libraries with antibody and protein probes," in *DNA Cloning 2: Expression Systems, 2nd Edition*, Glover *et al.* (eds.), pages 1-14 (Oxford University Press 1995)).

As an alternative, a *Zcytor21* gene can be obtained by synthesizing nucleic acid molecules using mutually priming long oligonucleotides and the nucleotide  
30 sequences described herein (see, for example, Ausubel (1995) at pages 8-8 to 8-9). Established techniques using the polymerase chain reaction provide the ability to

synthesize DNA molecules at least two kilobases in length (Adang *et al.*, *Plant Molec. Biol.* 21:1131 (1993), Bambot *et al.*, *PCR Methods and Applications* 2:266 (1993), Dillon *et al.*, "Use of the Polymerase Chain Reaction for the Rapid Construction of Synthetic Genes," in *Methods in Molecular Biology, Vol. 15: PCR Protocols: Current*  
5 *Methods and Applications*, White (ed.), pages 263-268, (Humana Press, Inc. 1993), and Holowachuk *et al.*, *PCR Methods Appl.* 4:299 (1995)).

The nucleic acid molecules of the present invention can also be synthesized with "gene machines" using protocols such as the phosphoramidite method. If chemically-synthesized double stranded DNA is required for an application such as the  
10 synthesis of a gene or a gene fragment, then each complementary strand is made separately. The production of short genes (60 to 80 base pairs) is technically straightforward and can be accomplished by synthesizing the complementary strands and then annealing them. For the production of longer genes (>300 base pairs), however, special strategies may be required, because the coupling efficiency of each cycle during  
15 chemical DNA synthesis is seldom 100%. To overcome this problem, synthetic genes (double-stranded) are assembled in modular form from single-stranded fragments that are from 20 to 100 nucleotides in length. For reviews on polynucleotide synthesis, see, for example, Glick and Pasternak, *Molecular Biotechnology, Principles and Applications of Recombinant DNA* (ASM Press 1994), Itakura *et al.*, *Annu. Rev. Biochem.* 53:323  
20 (1984), and Climie *et al.*, *Proc. Nat'l Acad. Sci. USA* 87:633 (1990).

The sequence of a *Zcytor21* cDNA or *Zcytor21* genomic fragment can be determined using standard methods. *Zcytor21* polynucleotide sequences disclosed herein can also be used as probes or primers to clone 5' non-coding regions of a *Zcytor21* gene. Promoter elements from a *Zcytor21* gene can be used to direct the expression of  
25 heterologous genes in, for example, transgenic animals or patients treated with gene therapy. The identification of genomic fragments containing a *Zcytor21* promoter or regulatory element can be achieved using well-established techniques, such as deletion analysis (see, generally, Ausubel (1995)).

Cloning of 5' flanking sequences also facilitates production of *Zcytor21*  
30 proteins by "gene activation," as disclosed in U.S. Patent No. 5,641,670. Briefly, expression of an endogenous *Zcytor21* gene in a cell is altered by introducing into the

*Zcytor21* locus a DNA construct comprising at least a targeting sequence, a regulatory sequence, an exon, and an unpaired splice donor site. The targeting sequence is a *Zcytor21* 5' non-coding sequence that permits homologous recombination of the construct with the endogenous *Zcytor21* locus, whereby the sequences within the  
5 construct become operably linked with the endogenous *Zcytor21* coding sequence. In this way, an endogenous *Zcytor21* promoter can be replaced or supplemented with other regulatory sequences to provide enhanced, tissue-specific, or otherwise regulated expression.

10 4. *Production of Zcytor21 Variants*

The present invention provides a variety of nucleic acid molecules, including DNA and RNA molecules, which encode the *Zcytor21* polypeptides disclosed herein. Those skilled in the art will readily recognize that, in view of the degeneracy of the genetic code, considerable sequence variation is possible among these polynucleotide  
15 molecules. For example, SEQ ID NO:12 is a degenerate nucleotide sequence that encompasses all nucleic acid molecules that encode the *Zcytor21*-d2 polypeptide of SEQ ID NO:11. Those skilled in the art will recognize that the degenerate sequence of SEQ ID NO:12 also provides all RNA sequences encoding SEQ ID NO:11, by substituting U for T. Thus, the present invention contemplates *Zcytor21*-d2 polypeptide-encoding  
20 nucleic acid molecules comprising nucleotide 66 to nucleotide 2066 of SEQ ID NO:10, and their RNA equivalents. Similarly, the present invention contemplates *Zcytor21* polypeptide-encoding nucleic acid molecules comprising nucleotide sequences that encode *Zcytor21*-f1, *Zcytor21*-f5, *Zcytor21*-f6, *Zcytor21*-g13, and their RNA equivalents.

25 Table 3 sets forth the one-letter codes used within SEQ ID NOs:3, 6, 9, 12, and 16 to denote degenerate nucleotide positions. "Resolutions" are the nucleotides denoted by a code letter. "Complement" indicates the code for the complementary nucleotide(s). For example, the code Y denotes either C or T, and its complement R denotes A or G, A being complementary to T, and G being complementary to C.

Table 3

Nucleotide	Resolution	Complement	Resolution
A	A	T	T
C	C	G	G
G	G	C	C
T	T	A	A
R	A G	Y	C T
Y	C T	R	A G
M	A C	K	G T
K	G T	M	A C
S	C G	S	C G
W	A T	W	A T
H	A C T	D	A G T
B	C G T	V	A C G
V	A C G	B	C G T
D	A G T	H	A C T
N	A C G T	N	A C G T

The degenerate codons used in SEQ ID NOs:3, 6, 9, 12, and 16,  
 5 encompassing all possible codons for a given amino acid, are set forth in Table 4.

Table 4

Amino Acid	One Letter Code	Codons	Degenerate Codon
Cys	C	TGC TGT	TGY
Ser	S	AGC AGT TCA TCC TCG TCT	WSN
Thr	T	ACA ACC ACG ACT	ACN
Pro	P	CCA CCC CCG CCT	CCN
Ala	A	GCA GCC GCG GCT	GCN
Gly	G	GGA GGC GGG GGT	GGN
Asn	N	AAC AAT	AAY
Asp	D	GAC GAT	GAY
Glu	E	GAA GAG	GAR
Gln	Q	CAA CAG	CAR
His	H	CAC CAT	CAY
Arg	R	AGA AGG CGA CGC CGG CGT	MGN
Lys	K	AAA AAG	AAR
Met	M	ATG	ATG
Ile	I	ATA ATC ATT	ATH
Leu	L	CTA CTC CTG CTT TTA TTG	YTN
Val	V	GTA GTC GTG GTT	GTN
Phe	F	TTC TTT	TTY
Tyr	Y	TAC TAT	TAY
Trp	W	TGG	TGG
Ter	.	TAA TAG TGA	TRR
Asn Asp	B		RAY
Glu Gln	Z		SAR
Any	X		NNN

One of ordinary skill in the art will appreciate that some ambiguity is introduced in determining a degenerate codon, representative of all possible codons encoding an amino acid. For example, the degenerate codon for serine (WSN) can, in some circumstances, encode arginine (AGR), and the degenerate codon for arginine  
5 (MGN) can, in some circumstances, encode serine (AGY). A similar relationship exists between codons encoding phenylalanine and leucine. Thus, some polynucleotides encompassed by the degenerate sequence may encode variant amino acid sequences, but one of ordinary skill in the art can easily identify such variant sequences by reference to the amino acid sequences of SEQ ID NOs:2, 5, 8, 11, and 15. Variant sequences can be  
10 readily tested for functionality as described herein.

Different species can exhibit "preferential codon usage." In general, see, Grantham *et al.*, *Nucl. Acids Res.* 8:1893 (1980), Haas *et al.* *Curr. Biol.* 6:315 (1996), Wain-Hobson *et al.*, *Gene* 13:355 (1981), Grosjean and Fiers, *Gene* 18:199 (1982), Holm, *Nucl. Acids Res.* 14:3075 (1986), Ikemura, *J. Mol. Biol.* 158:573 (1982), Sharp  
15 and Matassi, *Curr. Opin. Genet. Dev.* 4:851 (1994), Kane, *Curr. Opin. Biotechnol.* 6:494 (1995), and Makrides, *Microbiol. Rev.* 60:512 (1996). As used herein, the term "preferential codon usage" or "preferential codons" is a term of art referring to protein translation codons that are most frequently used in cells of a certain species, thus favoring one or a few representatives of the possible codons encoding each amino acid  
20 (See Table 4). For example, the amino acid threonine (Thr) may be encoded by ACA, ACC, ACG, or ACT, but in mammalian cells ACC is the most commonly used codon; in other species, for example, insect cells, yeast, viruses or bacteria, different Thr codons may be preferential. Preferential codons for a particular species can be introduced into the polynucleotides of the present invention by a variety of methods  
25 known in the art. Introduction of preferential codon sequences into recombinant DNA can, for example, enhance production of the protein by making protein translation more efficient within a particular cell type or species. Therefore, the degenerate codon sequences disclosed herein serve as a template for optimizing expression of polynucleotides in various cell-types and species commonly used in the art and  
30 disclosed herein. Sequences containing preferential codons can be tested and optimized for expression in various species, and tested for functionality as disclosed herein.



The present invention further provides variant polypeptides and nucleic acid molecules that represent counterparts from other species (orthologs). These species include, but are not limited to mammalian, avian, amphibian, reptile, fish, insect and other vertebrate and invertebrate species. Of particular interest are Zcytor21 polypeptides from other mammalian species, including mouse, porcine, ovine, bovine, canine, feline, equine, and other primate polypeptides. Orthologs of human Zcytor21 can be cloned using information and compositions provided by the present invention in combination with conventional cloning techniques. For example, a *Zcytor21* cDNA can be cloned using mRNA obtained from a tissue or cell type that expresses *Zcytor21* as disclosed herein. Suitable sources of mRNA can be identified by probing northern blots with probes designed from the sequences disclosed herein. A library is then prepared from mRNA of a positive tissue or cell line.

A *Zcytor21*-encoding cDNA can be isolated by a variety of methods, such as by probing with a complete or partial human cDNA or with one or more sets of degenerate probes based on the disclosed sequences. A cDNA can also be cloned using the polymerase chain reaction with primers designed from the representative human *Zcytor21* sequences disclosed herein. In addition, a cDNA library can be used to transform or transfect host cells, and expression of the cDNA of interest can be detected with an antibody to Zcytor21 polypeptide.

Those skilled in the art will recognize that the sequence disclosed in SEQ ID NO:10 represents a single allele of human *Zcytor21*, and that allelic variation and alternative splicing are expected to occur. Allelic variants of this sequence can be cloned by probing cDNA or genomic libraries from different individuals according to standard procedures. Allelic variants of the nucleotide sequences disclosed herein, including those containing silent mutations and those in which mutations result in amino acid sequence changes, are within the scope of the present invention, as are proteins which are allelic variants of the amino acid sequences disclosed herein. cDNA molecules generated from alternatively spliced mRNAs, which retain the properties of the Zcytor21 polypeptide are included within the scope of the present invention, as are polypeptides encoded by such cDNAs and mRNAs. Allelic variants and splice variants

of these sequences can be cloned by probing cDNA or genomic libraries from different individuals or tissues according to standard procedures known in the art.

Within certain embodiments of the invention, the isolated nucleic acid molecules can hybridize under stringent conditions to nucleic acid molecules comprising nucleotide sequences disclosed herein. Using Zcytor21-d2 as an example, such nucleic acid molecules can hybridize under stringent conditions to nucleic acid molecules comprising the nucleotide sequence of SEQ ID NO:10, to nucleic acid molecules consisting of the nucleotide sequence of nucleotides 66 to 2066 of SEQ ID NO:10, nucleotide sequence of nucleotides 135 to 1427 of SEQ ID NO:10, or to nucleic acid molecules comprising a nucleotide sequence complementary any of the nucleotide sequence of SEQ ID NO:10, the nucleotide sequence of nucleotides 66 to 2066 of SEQ ID NO:10, or nucleotides 135 to 1427 of SEQ ID NO:10. In general, stringent conditions are selected to be about 5°C lower than the thermal melting point ( $T_m$ ) for the specific sequence at a defined ionic strength and pH. The  $T_m$  is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe.

A pair of nucleic acid molecules, such as DNA-DNA, RNA-RNA and DNA-RNA, can hybridize if the nucleotide sequences have some degree of complementarity. Hybrids can tolerate mismatched base pairs in the double helix, but the stability of the hybrid is influenced by the degree of mismatch. The  $T_m$  of the mismatched hybrid decreases by 1°C for every 1-1.5% base pair mismatch. Varying the stringency of the hybridization conditions allows control over the degree of mismatch that will be present in the hybrid. The degree of stringency increases as the hybridization temperature increases and the ionic strength of the hybridization buffer decreases. Stringent hybridization conditions encompass temperatures of about 5°C - 25°C below the  $T_m$  of the hybrid and a hybridization buffer having up to 1 M  $\text{Na}^+$ . Higher degrees of stringency at lower temperatures can be achieved with the addition of formamide which reduces the  $T_m$  of the hybrid about 1°C for each 1% formamide in the buffer solution. Generally, such stringent conditions include temperatures of 20°C - 70°C and a hybridization buffer containing up to 6x SSC and 0-50% formamide. A higher degree of stringency can be achieved at temperatures of from 40°C - 70°C with a

hybridization buffer having up to 4x SSC and from 0-50% formamide. Highly stringent conditions typically encompass temperatures of 42°C - 70°C with a hybridization buffer having up to 1x SSC and 0-50% formamide. Different degrees of stringency can be used during hybridization and washing to achieve maximum specific binding to the target sequence. Typically, the washes following hybridization are performed at increasing degrees of stringency to remove non-hybridized polynucleotide probes from hybridized complexes.

The above conditions are meant to serve as a guide and it is well within the abilities of one skilled in the art to adapt these conditions for use with a particular polypeptide hybrid. The  $T_m$  for a specific target sequence is the temperature (under defined conditions) at which 50% of the target sequence will hybridize to a perfectly matched probe sequence. Conditions that influence the  $T_m$  include, the size and base pair content of the polynucleotide probe, the ionic strength of the hybridization solution, and the presence of destabilizing agents in the hybridization solution. Numerous equations for calculating  $T_m$  are known in the art, and are specific for DNA, RNA and DNA-RNA hybrids and polynucleotide probe sequences of varying length (see, for example, Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, Second Edition (Cold Spring Harbor Press 1989); Ausubel *et al.*, (eds.), *Current Protocols in Molecular Biology* (John Wiley and Sons, Inc. 1987); Berger and Kimmel (eds.), *Guide to Molecular Cloning Techniques*, (Academic Press, Inc. 1987); and Wetmur, *Crit. Rev. Biochem. Mol. Biol.* 26:227 (1990)). Sequence analysis software such as OLIGO 6.0 (LSR; Long Lake, MN) and *Primer Premier 4.0* (Premier Biosoft International; Palo Alto, CA), as well as sites on the Internet, are available tools for analyzing a given sequence and calculating  $T_m$  based on user defined criteria. Such programs can also analyze a given sequence under defined conditions and identify suitable probe sequences. Typically, hybridization of longer polynucleotide sequences, >50 base pairs, is performed at temperatures of about 20°C - 25°C below the calculated  $T_m$ . For smaller probes, <50 base pairs, hybridization is typically carried out at the  $T_m$  or 5°C - 10°C below. This allows for the maximum rate of hybridization for DNA-DNA and DNA-RNA hybrids.

The length of the polynucleotide sequence influences the rate and stability of hybrid formation. Smaller probe sequences, <50 base pairs, reach equilibrium with complementary sequences rapidly, but may form less stable hybrids. Incubation times of anywhere from minutes to hours can be used to achieve hybrid  
5 formation. Longer probe sequences come to equilibrium more slowly, but form more stable complexes even at lower temperatures. Incubations are allowed to proceed overnight or longer. Generally, incubations are carried out for a period equal to three times the calculated Cot time. Cot time, the time it takes for the polynucleotide sequences to reassociate, can be calculated for a particular sequence by methods known  
10 in the art.

The base pair composition of polynucleotide sequence will effect the thermal stability of the hybrid complex, thereby influencing the choice of hybridization temperature and the ionic strength of the hybridization buffer. A-T pairs are less stable than G-C pairs in aqueous solutions containing sodium chloride. Therefore, the higher  
15 the G-C content, the more stable the hybrid. Even distribution of G and C residues within the sequence also contribute positively to hybrid stability. In addition, the base pair composition can be manipulated to alter the  $T_m$  of a given sequence. For example, 5-methyldeoxycytidine can be substituted for deoxycytidine and 5-bromodeoxuridine can be substituted for thymidine to increase the  $T_m$ , whereas 7-deaz-2'-  
20 deoxyguanosine can be substituted for guanosine to reduce dependence on  $T_m$ .

The ionic concentration of the hybridization buffer also affects the stability of the hybrid. Hybridization buffers generally contain blocking agents such as Denhardt's solution (Sigma Chemical Co., St. Louis, Mo.), denatured salmon sperm DNA, tRNA, milk powders (BLOTTO), heparin or SDS, and a  $Na^+$  source, such as SSC  
25 (1x SSC: 0.15 M sodium chloride, 15 mM sodium citrate) or SSPE (1x SSPE: 1.8 M NaCl, 10 mM  $NaH_2PO_4$ , 1 mM EDTA, pH 7.7). Typically, hybridization buffers contain from between 10 mM - 1 M  $Na^+$ . The addition of destabilizing or denaturing agents such as formamide, tetraalkylammonium salts, guanidinium cations or thiocyanate cations to the hybridization solution will alter the  $T_m$  of a hybrid.  
30 Typically, formamide is used at a concentration of up to 50% to allow incubations to be

carried out at more convenient and lower temperatures. Formamide also acts to reduce non-specific background when using RNA probes.

As an illustration, a nucleic acid molecule encoding a variant Zcytor21 polypeptide can be hybridized with a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:10 (or its complement) at 42°C overnight in a solution comprising 50% formamide, 5x SSC, 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution (100x Denhardt's solution: 2% (w/v) Ficoll 400, 2% (w/v) polyvinylpyrrolidone, and 2% (w/v) bovine serum albumin), 10% dextran sulfate, and 20 µg/ml denatured, sheared salmon sperm DNA. One of skill in the art can devise variations of these hybridization conditions. For example, the hybridization mixture can be incubated at a higher temperature, such as about 65°C, in a solution that does not contain formamide. Moreover, premixed hybridization solutions are available (*e.g.*, EXPRESSHYB Hybridization Solution from CLONTECH Laboratories, Inc.), and hybridization can be performed according to the manufacturer's instructions.

Following hybridization, the nucleic acid molecules can be washed to remove non-hybridized nucleic acid molecules under stringent conditions, or under highly stringent conditions. Typical stringent washing conditions include washing in a solution of 0.5x - 2x SSC with 0.1% sodium dodecyl sulfate (SDS) at 55°C - 65°C. As an illustration, nucleic acid molecules encoding a variant Zcytor21-d2 polypeptide remain hybridized with a nucleic acid molecule comprising the nucleotide sequence of nucleotides 66 to 2066 of SEQ ID NO:10 (or its complement) under stringent washing conditions, in which the wash stringency is equivalent to 0.5x - 2x SSC with 0.1% SDS at 55°C - 65°C, including 0.5x SSC with 0.1% SDS at 55°C, or 2xSSC with 0.1% SDS at 65°C. One of skill in the art can readily devise equivalent conditions, for example, by substituting SSPE for SSC in the wash solution.

Typical highly stringent washing conditions include washing in a solution of 0.1x - 0.2x SSC with 0.1% sodium dodecyl sulfate (SDS) at 50°C - 65°C. For example, nucleic acid molecules encoding a variant Zcytor21-d2 polypeptide remain hybridized with a nucleic acid molecule comprising the nucleotide sequence of nucleotides 66 to 2066 of SEQ ID NO:10 (or its complement) under highly stringent washing conditions, in which the wash stringency is equivalent to 0.1x - 0.2x SSC with

0.1% SDS at 50°C - 65°C, including 0.1x SSC with 0.1% SDS at 50°C, or 0.2xSSC with 0.1% SDS at 65°C.

The present invention also provides isolated Zcytor21 polypeptides that have a substantially similar sequence identity to the polypeptides of SEQ ID NOs:2, 5, 8, 11, and 15, or their orthologs. The term "substantially similar sequence identity" is used herein to denote polypeptides having at least 70%, at least 80%, at least 90%, at least 95% or greater than 95% sequence identity to the sequences shown in SEQ ID NOs:2, 5, 8, 11, and 15, or their orthologs.

The present invention also contemplates *Zcytor21* variant nucleic acid molecules that can be identified using two criteria: a determination of the similarity between the encoded polypeptide with an amino acid sequence disclosed herein, and a hybridization assay, as described above. As an illustration, *Zcytor21-d2* variants include nucleic acid molecules (1) that remain hybridized with a nucleic acid molecule comprising the nucleotide sequence of nucleotides 66 to 2066 of SEQ ID NO:10 (or its complement) under stringent washing conditions, in which the wash stringency is equivalent to 0.5x - 2x SSC with 0.1% SDS at 55°C - 65°C, and (2) that encode a polypeptide having at least 70%, at least 80%, at least 90%, at least 95% or greater than 95% sequence identity to the amino acid sequence of SEQ ID NO:11. Alternatively, *Zcytor21-d2* variants can be characterized as nucleic acid molecules (1) that remain hybridized with a nucleic acid molecule comprising the nucleotide sequence of nucleotides 66 to 2066 of SEQ ID NO:10 (or its complement) under highly stringent washing conditions, in which the wash stringency is equivalent to 0.1x - 0.2x SSC with 0.1% SDS at 50°C - 65°C, and (2) that encode a polypeptide having at least 70%, at least 80%, at least 90%, at least 95% or greater than 95% sequence identity to the amino acid sequence of SEQ ID NO:11.

Percent sequence identity is determined by conventional methods. See, for example, Altschul *et al.*, *Bull. Math. Bio.* 48:603 (1986), and Henikoff and Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915 (1992). Briefly, two amino acid sequences are aligned to optimize the alignment scores using a gap opening penalty of 10, a gap extension penalty of 1, and the "BLOSUM62" scoring matrix of Henikoff and Henikoff (*ibid.*) as shown in Table 5 (amino acids are indicated by the standard one-

letter codes). The percent identity is then calculated as: 
$$\frac{[\text{Total number of identical matches}]}{[\text{length of the longer sequence plus the number of gaps introduced into the longer sequence in order to align the two sequences}]}(100).$$

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Table 5

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	4																			
R	-1	5																		
N	-2	0	6																	
D	-2	-2	1	6																
C	0	-3	-3	-3	9															
Q	-1	1	0	0	-3	5														
E	-1	0	0	2	-4	2	5													
G	0	-2	0	-1	-3	-2	-2	6												
H	-2	0	1	-1	-3	0	0	-2	8											
I	-1	-3	-3	-3	-1	-3	-4	-3	4											
L	-1	-2	-3	-4	-1	-2	-3	-4	-3	2	4									
K	-1	2	0	-1	-3	1	1	-2	-1	-3	-2	5								
M	-1	-1	-2	-3	-1	0	-2	-3	-2	1	2	-1	5							
F	-2	-3	-3	-3	-2	-3	-3	-3	-1	0	0	-3	0	6						
P	-1	-2	-2	-1	-3	-1	-1	-2	-2	-3	-3	-1	-2	-4	7					
S	1	-1	1	0	-1	0	0	0	-1	-2	-2	0	-1	-2	-1	4				
T	0	-1	0	-1	-1	-1	-1	-2	-2	-1	-1	-1	-2	-1	1	5				
W	-3	-3	-4	-4	-2	-2	-3	-2	-2	-3	-2	-3	-1	1	-4	-3	-2	11		
Y	-2	-2	-2	-3	-2	-1	-2	-3	2	-1	-1	-2	-1	3	-3	-2	-2	2	7	
V	0	-3	-3	-3	-1	-2	-2	-3	-3	3	1	-2	1	-1	-2	-2	0	-3	-1	4

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Those skilled in the art appreciate that there are many established algorithms available to align two amino acid sequences. The "FASTA" similarity search algorithm of Pearson and Lipman is a suitable protein alignment method for examining the level of identity shared by an amino acid sequence disclosed herein and the amino acid sequence of a putative Zcytor21 variant. The FASTA algorithm is described by Pearson and Lipman, *Proc. Nat'l Acad. Sci. USA* 85:2444 (1988), and by Pearson, *Meth. Enzymol.* 183:63 (1990). Briefly, FASTA first characterizes sequence similarity by identifying regions shared by the query sequence (*e.g.*, SEQ ID NO:11) and a test sequence that have either the highest density of identities (if the ktup variable is 1) or pairs of identities (if ktup=2), without considering conservative amino acid substitutions, insertions, or deletions. The ten regions with the highest density of identities are then rescored by comparing the similarity of all paired amino acids using an amino acid substitution matrix, and the ends of the regions are "trimmed" to include only those residues that contribute to the highest score. If there are several regions with scores greater than the "cutoff" value (calculated by a predetermined formula based upon the length of the sequence and the ktup value), then the trimmed initial regions are examined to determine whether the regions can be joined to form an approximate alignment with gaps. Finally, the highest scoring regions of the two amino acid sequences are aligned using a modification of the Needleman-Wunsch-Sellers algorithm (Needleman and Wunsch, *J. Mol. Biol.* 48:444 (1970); Sellers, *SIAM J. Appl. Math.* 26:787 (1974)), which allows for amino acid insertions and deletions. Illustrative parameters for FASTA analysis are: ktup=1, gap opening penalty=10, gap extension penalty=1, and substitution matrix=BLOSUM62. These parameters can be introduced into a FASTA program by modifying the scoring matrix file ("SMATRIX"), as explained in Appendix 2 of Pearson, *Meth. Enzymol.* 183:63 (1990).

FASTA can also be used to determine the sequence identity of nucleic acid molecules using a ratio as disclosed above. For nucleotide sequence comparisons, the ktup value can range between one to six, preferably from three to six, most preferably three, with other parameters set as described above.

The present invention includes nucleic acid molecules that encode a polypeptide having a conservative amino acid change, compared with an amino acid

sequence disclosed herein. For example, variants can be obtained that contain one or more amino acid substitutions of SEQ ID NOs:2, 5, 8, 11, or 15, in which an alkyl amino acid is substituted for an alkyl amino acid in a Zcytor21 amino acid sequence, an aromatic amino acid is substituted for an aromatic amino acid in a Zcytor21 amino acid sequence, a sulfur-containing amino acid is substituted for a sulfur-containing amino acid in a Zcytor21 amino acid sequence, a hydroxy-containing amino acid is substituted for a hydroxy-containing amino acid in a Zcytor21 amino acid sequence, an acidic amino acid is substituted for an acidic amino acid in a Zcytor21 amino acid sequence, a basic amino acid is substituted for a basic amino acid in a Zcytor21 amino acid sequence, or a dibasic monocarboxylic amino acid is substituted for a dibasic monocarboxylic amino acid in a Zcytor21 amino acid sequence. Among the common amino acids, for example, a "conservative amino acid substitution" is illustrated by a substitution among amino acids within each of the following groups: (1) glycine, alanine, valine, leucine, and isoleucine, (2) phenylalanine, tyrosine, and tryptophan, (3) serine and threonine, (4) aspartate and glutamate, (5) glutamine and asparagine, and (6) lysine, arginine and histidine.

The BLOSUM62 table is an amino acid substitution matrix derived from about 2,000 local multiple alignments of protein sequence segments, representing highly conserved regions of more than 500 groups of related proteins (Henikoff and Henikoff, *Proc. Nat'l Acad. Sci. USA* 89:10915 (1992)). Accordingly, the BLOSUM62 substitution frequencies can be used to define conservative amino acid substitutions that may be introduced into the amino acid sequences of the present invention. Although it is possible to design amino acid substitutions based solely upon chemical properties (as discussed above), the language "conservative amino acid substitution" preferably refers to a substitution represented by a BLOSUM62 value of greater than -1. For example, an amino acid substitution is conservative if the substitution is characterized by a BLOSUM62 value of 0, 1, 2, or 3. According to this system, preferred conservative amino acid substitutions are characterized by a BLOSUM62 value of at least 1 (e.g., 1, 2 or 3), while more preferred conservative amino acid substitutions are characterized by a BLOSUM62 value of at least 2 (e.g., 2 or 3).

Particular variants of Zcytor21 are characterized by having at least 70%, at least 80%, at least 90%, at least 95% or greater than 95% sequence identity to the corresponding amino acid sequence (e.g., SEQ ID NOs:2, 5, 8, 11, or 15), wherein the variation in amino acid sequence is due to one or more conservative amino acid substitutions.

Conservative amino acid changes in a *Zcytor21* gene can be introduced, for example, by substituting nucleotides for the nucleotides recited in SEQ ID NO:10. Such "conservative amino acid" variants can be obtained by oligonucleotide-directed mutagenesis, linker-scanning mutagenesis, mutagenesis using the polymerase chain reaction, and the like (see Ausubel (1995) at pages 8-10 to 8-22; and McPherson (ed.), *Directed Mutagenesis: A Practical Approach* (IRL Press 1991)). A variant *Zcytor21* polypeptide can be identified by the ability to specifically bind anti-*Zcytor21* antibodies.

The proteins of the present invention can also comprise non-naturally occurring amino acid residues. Non-naturally occurring amino acids include, without limitation, *trans*-3-methylproline, 2,4-methanoproline, *cis*-4-hydroxyproline, *trans*-4-hydroxyproline, *N*-methylglycine, *allo*-threonine, methylthreonine, hydroxyethylcysteine, hydroxyethylhomocysteine, nitroglutamine, homoglutamine, pipecolic acid, thiazolidine carboxylic acid, dehydropyrolidine, 3- and 4-methylproline, 3,3-dimethylproline, *tert*-leucine, norvaline, 2-azaphenylalanine, 3-azaphenylalanine, 4-azaphenylalanine, and 4-fluorophenylalanine. Several methods are known in the art for incorporating non-naturally occurring amino acid residues into proteins. For example, an *in vitro* system can be employed wherein nonsense mutations are suppressed using chemically aminoacylated suppressor tRNAs. Methods for synthesizing amino acids and aminoacylating tRNA are known in the art. Transcription and translation of plasmids containing nonsense mutations is typically carried out in a cell-free system comprising an *E. coli* S30 extract and commercially available enzymes and other reagents. Proteins are purified by chromatography. See, for example, Robertson *et al.*, *J. Am. Chem. Soc.* 113:2722 (1991), Ellman *et al.*, *Methods Enzymol.* 202:301 (1991), Chung *et al.*, *Science* 259:806 (1993), and Chung *et al.*, *Proc. Nat'l Acad. Sci. USA* 90:10145 (1993).

In a second method, translation is carried out in *Xenopus* oocytes by microinjection of mutated mRNA and chemically aminoacylated suppressor tRNAs (Turcatti *et al.*, *J. Biol. Chem.* 271:19991 (1996)). Within a third method, *E. coli* cells are cultured in the absence of a natural amino acid that is to be replaced (e.g., phenylalanine) and in the presence of the desired non-naturally occurring amino acid(s) (e.g., 2-azaphenylalanine, 3-azaphenylalanine, 4-azaphenylalanine, or 4-fluorophenylalanine). The non-naturally occurring amino acid is incorporated into the protein in place of its natural counterpart. See, Koide *et al.*, *Biochem.* 33:7470 (1994). Naturally occurring amino acid residues can be converted to non-naturally occurring species by *in vitro* chemical modification. Chemical modification can be combined with site-directed mutagenesis to further expand the range of substitutions (Wynn and Richards, *Protein Sci.* 2:395 (1993)).

A limited number of non-conservative amino acids, amino acids that are not encoded by the genetic code, non-naturally occurring amino acids, and unnatural amino acids may be substituted for Zcytor21 amino acid residues.

Essential amino acids in the polypeptides of the present invention can be identified according to procedures known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, *Science* 244:1081 (1989), Bass *et al.*, *Proc. Nat'l Acad. Sci. USA* 88:4498 (1991), Coombs and Corey, "Site-Directed Mutagenesis and Protein Engineering," in *Proteins: Analysis and Design*, Angeletti (ed.), pages 259-311 (Academic Press, Inc. 1998)). In the latter technique, single alanine mutations are introduced at every residue in the molecule, and the resultant mutant molecules are tested for biological activity to identify amino acid residues that are critical to the activity of the molecule. See also, Hilton *et al.*, *J. Biol. Chem.* 271:4699 (1996).

Although sequence analysis can be used to further define the Zcytor21 ligand binding region, amino acids that play a role in Zcytor21 binding activity can also be determined by physical analysis of structure, as determined by such techniques as nuclear magnetic resonance, crystallography, electron diffraction or photoaffinity labeling, in conjunction with mutation of putative contact site amino acids. See, for

example, de Vos *et al.*, *Science* 255:306 (1992), Smith *et al.*, *J. Mol. Biol.* 224:899 (1992), and Wlodaver *et al.*, *FEBS Lett.* 309:59 (1992).

Multiple amino acid substitutions can be made and tested using known methods of mutagenesis and screening, such as those disclosed by Reidhaar-Olson and Sauer (*Science* 241:53 (1988)) or Bowie and Sauer (*Proc. Nat'l Acad. Sci. USA* 86:2152 (1989)). Briefly, these authors disclose methods for simultaneously randomizing two or more positions in a polypeptide, selecting for functional polypeptide, and then sequencing the mutagenized polypeptides to determine the spectrum of allowable substitutions at each position. Other methods that can be used include phage display (*e.g.*, Lowman *et al.*, *Biochem.* 30:10832 (1991), Ladner *et al.*, U.S. Patent No. 5,223,409, Huse, international publication No. WO 92/06204, and region-directed mutagenesis (Derbyshire *et al.*, *Gene* 46:145 (1986), and Ner *et al.*, *DNA* 7:127, (1988)). Moreover, Zcytor21 labeled with biotin or FITC can be used for expression cloning of Zcytor21 ligands.

Variants of the disclosed Zcytor21 nucleotide and polypeptide sequences can also be generated through DNA shuffling as disclosed by Stemmer, *Nature* 370:389 (1994), Stemmer, *Proc. Nat'l Acad. Sci. USA* 91:10747 (1994), and international publication No. WO 97/20078. Briefly, variant DNA molecules are generated by *in vitro* homologous recombination by random fragmentation of a parent DNA followed by reassembly using PCR, resulting in randomly introduced point mutations. This technique can be modified by using a family of parent DNA molecules, such as allelic variants or DNA molecules from different species, to introduce additional variability into the process. Selection or screening for the desired activity, followed by additional iterations of mutagenesis and assay provides for rapid "evolution" of sequences by selecting for desirable mutations while simultaneously selecting against detrimental changes.

Mutagenesis methods as disclosed herein can be combined with high-throughput, automated screening methods to detect activity of cloned, mutagenized polypeptides in host cells. Mutagenized DNA molecules that encode biologically active polypeptides, or polypeptides that bind with anti-Zcytor21 antibodies, can be recovered from the host cells and rapidly sequenced using modern equipment. These methods

allow the rapid determination of the importance of individual amino acid residues in a polypeptide of interest, and can be applied to polypeptides of unknown structure.

The present invention also includes "functional fragments" of Zcytor21 polypeptides and nucleic acid molecules encoding such functional fragments. Routine deletion analyses of nucleic acid molecules can be performed to obtain functional fragments of a nucleic acid molecule that encodes a Zcytor21 polypeptide. As an illustration, DNA molecules comprising the nucleotide sequence of nucleotides 66 to 2066 of SEQ ID NO:10 can be digested with *Bal31* nuclease to obtain a series of nested deletions. The fragments are then inserted into expression vectors in proper reading frame, and the expressed polypeptides are isolated and tested for the ability to bind anti-Zcytor21 antibodies. One alternative to exonuclease digestion is to use oligonucleotide-directed mutagenesis to introduce deletions or stop codons to specify production of a desired fragment. Alternatively, particular fragments of a *Zcytor21* gene can be synthesized using the polymerase chain reaction. An example of a functional fragment is the extracellular domain of Zcytor21 (*i.e.*, amino acid residues 24 to 454 of SEQ ID NO:11, amino acid residues 24 to 376 of SEQ ID NO:2, amino acid residues 24 to 396 of SEQ ID NO:5, amino acid residues 24 to 533 of SEQ ID NO:8, or amino acid residues 24 to 444 of SEQ ID NO:15).

This general approach is exemplified by studies on the truncation at either or both termini of interferons have been summarized by Horisberger and Di Marco, *Pharmac. Ther.* 66:507 (1995). Moreover, standard techniques for functional analysis of proteins are described by, for example, Treuter *et al.*, *Molec. Gen. Genet.* 240:113 (1993), Content *et al.*, "Expression and preliminary deletion analysis of the 42 kDa 2-5A synthetase induced by human interferon," in *Biological Interferon Systems, Proceedings of ISIR-TNO Meeting on Interferon Systems*, Cantell (ed.), pages 65-72 (Nijhoff 1987), Herschman, "The EGF Receptor," in *Control of Animal Cell Proliferation, Vol. 1*, Boynton *et al.*, (eds.) pages 169-199 (Academic Press 1985), Coumailleau *et al.*, *J. Biol. Chem.* 270:29270 (1995); Fukunaga *et al.*, *J. Biol. Chem.* 270:25291 (1995); Yamaguchi *et al.*, *Biochem. Pharmacol.* 50:1295 (1995), and Meisel *et al.*, *Plant Molec. Biol.* 30:1 (1996).

The present invention also contemplates functional fragments of a *Zcytor21* gene that have amino acid changes, compared with an amino acid sequence disclosed herein. A variant *Zcytor21* gene can be identified on the basis of structure by determining the level of identity with disclosed nucleotide and amino acid sequences, as discussed above. An alternative approach to identifying a variant gene on the basis of structure is to determine whether a nucleic acid molecule encoding a potential variant *Zcytor21* gene can hybridize to a nucleic acid molecule comprising a *Zcytor21* nucleotide sequence, such as SEQ ID NO:10.

The present invention also provides polypeptide fragments or peptides comprising an epitope-bearing portion of a *Zcytor21* polypeptide described herein. Such fragments or peptides may comprise an "immunogenic epitope," which is a part of a protein that elicits an antibody response when the entire protein is used as an immunogen. Immunogenic epitope-bearing peptides can be identified using standard methods (see, for example, Geysen *et al.*, *Proc. Nat'l Acad. Sci. USA* 81:3998 (1983)).

In contrast, polypeptide fragments or peptides may comprise an "antigenic epitope," which is a region of a protein molecule to which an antibody can specifically bind. Certain epitopes consist of a linear or contiguous stretch of amino acids, and the antigenicity of such an epitope is not disrupted by denaturing agents. It is known in the art that relatively short synthetic peptides that can mimic epitopes of a protein can be used to stimulate the production of antibodies against the protein (see, for example, Sutcliffe *et al.*, *Science* 219:660 (1983)). Accordingly, antigenic epitope-bearing peptides and polypeptides of the present invention are useful to raise antibodies that bind with the polypeptides described herein.

Antigenic epitope-bearing peptides and polypeptides can contain at least four to ten amino acids, at least ten to fifteen amino acids, or about 15 to about 30 amino acids of an amino acid sequence disclosed herein. Such epitope-bearing peptides and polypeptides can be produced by fragmenting a *Zcytor21* polypeptide, or by chemical peptide synthesis, as described herein. Moreover, epitopes can be selected by phage display of random peptide libraries (see, for example, Lane and Stephen, *Curr. Opin. Immunol.* 5:268 (1993), and Cortese *et al.*, *Curr. Opin. Biotechnol.* 7:616 (1996)). Standard methods for identifying epitopes and producing antibodies from

small peptides that comprise an epitope are described, for example, by Mole, "Epitope Mapping," in *Methods in Molecular Biology*, Vol. 10, Manson (ed.), pages 105-116 (The Humana Press, Inc. 1992), Price, "Production and Characterization of Synthetic Peptide-Derived Antibodies," in *Monoclonal Antibodies: Production, Engineering, and Clinical Application*, Ritter and Ladyman (eds.), pages 60-84 (Cambridge University Press 1995), and Coligan *et al.* (eds.), *Current Protocols in Immunology*, pages 9.3.1 - 9.3.5 and pages 9.4.1 - 9.4.11 (John Wiley & Sons 1997).

In addition to the uses described above, polynucleotides and polypeptides of the present invention are useful as educational tools in laboratory practicum kits for courses related to genetics and molecular biology, protein chemistry, and antibody production and analysis. Due to its unique polynucleotide and polypeptide sequences, molecules of Zcytor21 can be used as standards or as "unknowns" for testing purposes. Advantageously, the present invention provides four Zcytor21 variants suitable for analysis. As an illustration, Zcytor21 polynucleotides can be used as an aid, such as, for example, to teach a student how to prepare expression constructs for bacterial, viral, or mammalian expression, including fusion constructs, wherein Zcytor21 is the gene to be expressed; for determining the restriction endonuclease cleavage sites of the polynucleotides; determining mRNA and DNA localization of Zcytor21 polynucleotides in tissues (*i.e.*, by northern and Southern blotting as well as polymerase chain reaction); and for identifying related polynucleotides and polypeptides by nucleic acid hybridization. As an illustration, students will find that *Bam*HI digestion of a nucleic acid molecule consisting of the nucleotide sequence of nucleotides 66 to 2066 of SEQ ID NO:10 provides two fragments of about 475 base pairs, and 1526 base pairs, and that *Xho*I digestion yields fragments of about 963 base pairs, and 1038 base pairs.

Zcytor21 polypeptides can be used as an aid to teach preparation of antibodies; identifying proteins by western blotting; protein purification; determining the weight of expressed Zcytor21 polypeptides as a ratio to total protein expressed; identifying peptide cleavage sites; coupling amino and carboxyl terminal tags; amino acid sequence analysis, as well as, but not limited to monitoring biological activities of both the native and tagged protein (*i.e.*, protease inhibition) *in vitro* and *in vivo*. For



example, students will find that digestion of unglycosylated Zcytor21 with hydroxylamine yields two fragments having approximate molecular weights of 36361, and 38465, whereas digestion of unglycosylated Zcytor21 with mild acid hydrolysis yields fragments having approximate molecular weights of 18042, 45959, and 10842.

5                   Zcytor21 polypeptides can also be used to teach analytical skills such as mass spectrometry, circular dichroism, to determine conformation, especially of the four alpha helices, x-ray crystallography to determine the three-dimensional structure in atomic detail, nuclear magnetic resonance spectroscopy to reveal the structure of proteins in solution. For example, a kit containing the Zcytor21 can be given to the student to analyze. Since the amino acid sequence would be known by the instructor, the protein can be given to the student as a test to determine the skills or develop the skills of the student, the instructor would then know whether or not the student has correctly analyzed the polypeptide. Since every polypeptide is unique, the educational utility of Zcytor21 would be unique unto itself.

15                   The antibodies which bind specifically to Zcytor21 can be used as a teaching aid to instruct students how to prepare affinity chromatography columns to purify Zcytor21, cloning and sequencing the polynucleotide that encodes an antibody and thus as a practicum for teaching a student how to design humanized antibodies. The Zcytor21 gene, polypeptide, or antibody would then be packaged by reagent companies and sold to educational institutions so that the students gain skill in art of molecular biology. Because each gene and protein is unique, each gene and protein creates unique challenges and learning experiences for students in a lab practicum. Such educational kits containing the Zcytor21 gene, polypeptide, or antibody are considered within the scope of the present invention.

25                   For any Zcytor21 polypeptide, including variants and fusion proteins, one of ordinary skill in the art can readily generate a fully degenerate polynucleotide sequence encoding that variant using the information set forth in Tables 3 and 4 above. Moreover, those of skill in the art can use standard software to devise Zcytor21 variants based upon the nucleotide and amino acid sequences described herein. Accordingly, the present invention includes a computer-readable medium encoded with a data structure that provides at least one of the following sequences: SEQ ID NO:1, SEQ ID

30

NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:15, and SEQ ID NO:16. Suitable forms of computer-readable media include magnetic media and optically-readable media. Examples of magnetic media include a hard or fixed drive, a random access memory (RAM) chip, a floppy disk, digital linear tape (DLT), a disk cache, and a ZIP disk. Optically readable media are exemplified by compact discs (*e.g.*, CD-read only memory (ROM), CD-rewritable (RW), and CD-recordable), and digital versatile/video discs (DVD) (*e.g.*, DVD-ROM, DVD-RAM, and DVD+RW).

#### 5. *Production of Zcytor21 Polypeptides*

The polypeptides of the present invention, including full-length polypeptides, functional fragments, and fusion proteins, can be produced in recombinant host cells following conventional techniques. To express a *Zcytor21* gene, a nucleic acid molecule encoding the polypeptide must be operably linked to regulatory sequences that control transcriptional expression in an expression vector and then, introduced into a host cell. In addition to transcriptional regulatory sequences, such as promoters and enhancers, expression vectors can include translational regulatory sequences and a marker gene, which is suitable for selection of cells that carry the expression vector.

Expression vectors that are suitable for production of a foreign protein in eukaryotic cells typically contain (1) prokaryotic DNA elements coding for a bacterial replication origin and an antibiotic resistance marker to provide for the growth and selection of the expression vector in a bacterial host; (2) eukaryotic DNA elements that control initiation of transcription, such as a promoter; and (3) DNA elements that control the processing of transcripts, such as a transcription termination/polyadenylation sequence. As discussed above, expression vectors can also include nucleotide sequences encoding a secretory sequence that directs the heterologous polypeptide into the secretory pathway of a host cell. For example, a *Zcytor21* expression vector may comprise a *Zcytor21* gene and a secretory sequence derived from any secreted gene.

*Zcytor21* proteins of the present invention may be expressed in mammalian cells. Examples of suitable mammalian host cells include African green

monkey kidney cells (Vero; ATCC CRL 1587), human embryonic kidney cells (293-HEK; ATCC CRL 1573), baby hamster kidney cells (BHK-21, BHK-570; ATCC CRL 8544, ATCC CRL 10314), canine kidney cells (MDCK; ATCC CCL 34), Chinese hamster ovary cells (CHO-K1; ATCC CCL61; CHO DG44 (Chasin *et al.*, *Som. Cell. Molec. Genet.* 12:555, 1986)), rat pituitary cells (GH1; ATCC CCL82), HeLa S3 cells (ATCC CCL2.2), rat hepatoma cells (H-4-II-E; ATCC CRL 1548) SV40-transformed monkey kidney cells (COS-1; ATCC CRL 1650) and murine embryonic cells (NIH-3T3; ATCC CRL 1658).

For a mammalian host, the transcriptional and translational regulatory signals may be derived from viral sources, such as adenovirus, bovine papilloma virus, simian virus, or the like, in which the regulatory signals are associated with a particular gene which has a high level of expression. Suitable transcriptional and translational regulatory sequences also can be obtained from mammalian genes, such as actin, collagen, myosin, and metallothionein genes.

Transcriptional regulatory sequences include a promoter region sufficient to direct the initiation of RNA synthesis. Suitable eukaryotic promoters include the promoter of the mouse *metallothionein I* gene (Hamer *et al.*, *J. Molec. Appl. Genet.* 1:273 (1982)), the *TK* promoter of *Herpes* virus (McKnight, *Cell* 31:355 (1982)), the *SV40* early promoter (Benoist *et al.*, *Nature* 290:304 (1981)), the *Rous* sarcoma virus promoter (Gorman *et al.*, *Proc. Nat'l Acad. Sci. USA* 79:6777 (1982)), the cytomegalovirus promoter (Foecking *et al.*, *Gene* 45:101 (1980)), and the mouse mammary tumor virus promoter (see, generally, Etcheverry, "Expression of Engineered Proteins in Mammalian Cell Culture," in *Protein Engineering: Principles and Practice*, Cleland *et al.* (eds.), pages 163-181 (John Wiley & Sons, Inc. 1996)).

Alternatively, a prokaryotic promoter, such as the bacteriophage T3 RNA polymerase promoter, can be used to control *Zcytor21* gene expression in mammalian cells if the prokaryotic promoter is regulated by a eukaryotic promoter (Zhou *et al.*, *Mol. Cell. Biol.* 10:4529 (1990), and Kaufman *et al.*, *Nucl. Acids Res.* 19:4485 (1991)).

An expression vector can be introduced into host cells using a variety of standard techniques including calcium phosphate transfection, liposome-mediated

transfection, microprojectile-mediated delivery, electroporation, and the like. The transfected cells can be selected and propagated to provide recombinant host cells that comprise the expression vector stably integrated in the host cell genome. Techniques for introducing vectors into eukaryotic cells and techniques for selecting such stable transformants using a dominant selectable marker are described, for example, by Ausubel  
5 (1995) and by Murray (ed.), *Gene Transfer and Expression Protocols* (Humana Press 1991).

For example, one suitable selectable marker is a gene that provides resistance to the antibiotic neomycin. In this case, selection is carried out in the presence of a neomycin-type drug, such as G-418 or the like. Selection systems can also be used to increase the expression level of the gene of interest, a process referred to as "amplification." Amplification is carried out by culturing transfectants in the presence of a low level of the selective agent and then increasing the amount of selective agent to select for cells that produce high levels of the products of the introduced genes. A suitable amplifiable selectable marker is dihydrofolate reductase,  
15 which confers resistance to methotrexate. Other drug resistance genes (*e.g.*, hygromycin resistance, multi-drug resistance, puromycin acetyltransferase) can also be used. Alternatively, markers that introduce an altered phenotype, such as green fluorescent protein, or cell surface proteins such as CD4, CD8, Class I MHC, placental alkaline phosphatase may be used to sort transfected cells from untransfected cells by  
20 such means as FACS sorting or magnetic bead separation technology.

Zcytor21 polypeptides can also be produced by cultured mammalian cells using a viral delivery system. Exemplary viruses for this purpose include adenovirus, herpesvirus, vaccinia virus and adeno-associated virus (AAV).  
25 Adenovirus, a double-stranded DNA virus, is currently the best studied gene transfer vector for delivery of heterologous nucleic acid (for a review, see Becker *et al.*, *Meth. Cell Biol.* 43:161 (1994), and Douglas and Curiel, *Science & Medicine* 4:44 (1997)). Advantages of the adenovirus system include the accommodation of relatively large DNA inserts, the ability to grow to high-titer, the ability to infect a broad range of mammalian cell types, and flexibility that allows use with a large number of available  
30 vectors containing different promoters.

By deleting portions of the adenovirus genome, larger inserts (up to 7 kb) of heterologous DNA can be accommodated. These inserts can be incorporated into the viral DNA by direct ligation or by homologous recombination with a co-transfected plasmid. An option is to delete the essential *E1* gene from the viral vector, which results in the inability to replicate unless the *E1* gene is provided by the host cell. Adenovirus vector-infected human 293 cells (ATCC Nos. CRL-1573, 45504, 45505), for example, can be grown as adherent cells or in suspension culture at relatively high cell density to produce significant amounts of protein (see Garnier *et al.*, *Cytotechnol.* 15:145 (1994)).

Zcytor21 can also be expressed in other higher eukaryotic cells, such as avian, fungal, insect, yeast, or plant cells. The baculovirus system provides an efficient means to introduce cloned *Zcytor21* genes into insect cells. Suitable expression vectors are based upon the *Autographa californica* multiple nuclear polyhedrosis virus (AcMNPV), and contain well-known promoters such as *Drosophila heat shock protein* (*hsp*) 70 promoter, *Autographa californica* nuclear polyhedrosis virus immediate-early gene promoter (*ie-1*) and the *delayed early* 39K promoter, baculovirus *p10* promoter, and the *Drosophila metallothionein* promoter. A second method of making recombinant baculovirus utilizes a transposon-based system described by Luckow (Luckow, *et al.*, *J. Virol.* 67:4566 (1993)). This system, which utilizes transfer vectors, is sold in the BAC-to-BAC kit (Life Technologies, Rockville, MD). This system utilizes a transfer vector, PFASTBAC (Life Technologies) containing a Tn7 transposon to move the DNA encoding the *Zcytor21* polypeptide into a baculovirus genome maintained in *E. coli* as a large plasmid called a "bacmid." See, Hill-Perkins and Possee, *J. Gen. Virol.* 71:971 (1990), Bonning, *et al.*, *J. Gen. Virol.* 75:1551 (1994), and Chazenbalk, and Rapoport, *J. Biol. Chem.* 270:1543 (1995). In addition, transfer vectors can include an in-frame fusion with DNA encoding an epitope tag at the C- or N-terminus of the expressed *Zcytor21* polypeptide, for example, a Glu-Glu epitope tag (Grussenmeyer *et al.*, *Proc. Nat'l Acad. Sci.* 82:7952 (1985)). Using a technique known in the art, a transfer vector containing a *Zcytor21* gene is transformed into *E. coli*, and screened for bacmids, which contain an interrupted *lacZ* gene indicative of recombinant baculovirus. The bacmid

DNA containing the recombinant baculovirus genome is then isolated using common techniques.

The illustrative PFASTBAC vector can be modified to a considerable degree. For example, the polyhedrin promoter can be removed and substituted with the baculovirus basic protein promoter (also known as *Pcor*, p6.9 or MP promoter) which is expressed earlier in the baculovirus infection, and has been shown to be advantageous for expressing secreted proteins (see, for example, Hill-Perkins and Possee, *J. Gen. Virol.* 71:971 (1990), Bonning, *et al.*, *J. Gen. Virol.* 75:1551 (1994), and Chazenbalk and Rapoport, *J. Biol. Chem.* 270:1543 (1995). In such transfer vector constructs, a short or long version of the basic protein promoter can be used. Moreover, transfer vectors can be constructed, which replace the native *Zcytor21* secretory signal sequences with secretory signal sequences derived from insect proteins. For example, a secretory signal sequence from Ecdysteroid Glucosyltransferase (EGT), honey bee Melittin (Invitrogen Corporation; Carlsbad, CA), or baculovirus gp67 (PharMingen: San Diego, CA) can be used in constructs to replace the native *Zcytor21* secretory signal sequence.

The recombinant virus or bacmid is used to transfect host cells. Suitable insect host cells include cell lines derived from IPLB-Sf-21, a *Spodoptera frugiperda* pupal ovarian cell line, such as Sf9 (ATCC CRL 1711), Sf21AE, and Sf21 (Invitrogen Corporation; San Diego, CA), as well as *Drosophila* Schneider-2 cells, and the HIGH FIVEO cell line (Invitrogen) derived from *Trichoplusia ni* (U.S. Patent No. 5,300,435). Commercially available serum-free media can be used to grow and to maintain the cells. Suitable media are Sf900 II™ (Life Technologies) or ESF 921™ (Expression Systems) for the Sf9 cells; and Ex-cello405™ (JRH Biosciences, Lenexa, KS) or Express FiveO™ (Life Technologies) for the *T. ni* cells. When recombinant virus is used, the cells are typically grown up from an inoculation density of approximately  $2.5 \times 10^5$  cells to a density of  $1-2 \times 10^6$  cells at which time a recombinant viral stock is added at a multiplicity of infection (MOI) of 0.1 to 10, more typically near 3.

Established techniques for producing recombinant proteins in baculovirus systems are provided by Bailey *et al.*, "Manipulation of Baculovirus Vectors," in *Methods in Molecular Biology, Volume 7: Gene Transfer and Expression*

*Protocols*, Murray (ed.), pages 147-168 (The Humana Press, Inc. 1991), by Patel *et al.*, "The baculovirus expression system," in *DNA Cloning 2: Expression Systems, 2nd Edition*, Glover *et al.* (eds.), pages 205-244 (Oxford University Press 1995), by Ausubel (1995) at pages 16-37 to 16-57, by Richardson (ed.), *Baculovirus Expression Protocols* (The Humana Press, Inc. 1995), and by Lucknow, "Insect Cell Expression Technology," in *Protein Engineering: Principles and Practice*, Cleland *et al.* (eds.), pages 183-218 (John Wiley & Sons, Inc. 1996).

Fungal cells, including yeast cells, can also be used to express the genes described herein. Yeast species of particular interest in this regard include *Saccharomyces cerevisiae*, *Pichia pastoris*, and *Pichia methanolica*. Suitable promoters for expression in yeast include promoters from *GALI* (galactose), *PGK* (phosphoglycerate kinase), *ADH* (alcohol dehydrogenase), *AOXI* (alcohol oxidase), *HIS4* (histidinol dehydrogenase), and the like. Many yeast cloning vectors have been designed and are readily available. These vectors include YIp-based vectors, such as YIp5, YRp vectors, such as YRp17, YE<sub>p</sub> vectors such as YE<sub>p</sub>13 and YC<sub>p</sub> vectors, such as YC<sub>p</sub>19. Methods for transforming *S. cerevisiae* cells with exogenous DNA and producing recombinant polypeptides therefrom are disclosed by, for example, Kawasaki, U.S. Patent No. 4,599,311, Kawasaki *et al.*, U.S. Patent No. 4,931,373, Brake, U.S. Patent No. 4,870,008, Welch *et al.*, U.S. Patent No. 5,037,743, and Murray *et al.*, U.S. Patent No. 4,845,075. Transformed cells are selected by phenotype determined by the selectable marker, commonly drug resistance or the ability to grow in the absence of a particular nutrient (*e.g.*, leucine). A suitable vector system for use in *Saccharomyces cerevisiae* is the *POT1* vector system disclosed by Kawasaki *et al.* (U.S. Patent No. 4,931,373), which allows transformed cells to be selected by growth in glucose-containing media. Additional suitable promoters and terminators for use in yeast include those from glycolytic enzyme genes (see, *e.g.*, Kawasaki, U.S. Patent No. 4,599,311, Kingsman *et al.*, U.S. Patent No. 4,615,974, and Bitter, U.S. Patent No. 4,977,092) and alcohol dehydrogenase genes. See also U.S. Patents Nos. 4,990,446, 5,063,154, 5,139,936, and 4,661,454.

Transformation systems for other yeasts, including *Hansenula polymorpha*, *Schizosaccharomyces pombe*, *Kluyveromyces lactis*, *Kluyveromyces*

*fragilis*, *Ustilago maydis*, *Pichia pastoris*, *Pichia methanolica*, *Pichia guilliermondii* and *Candida maltosa* are known in the art. See, for example, Gleeson *et al.*, *J. Gen. Microbiol.* 132:3459 (1986), and Cregg, U.S. Patent No. 4,882,279. *Aspergillus* cells may be utilized according to the methods of McKnight *et al.*, U.S. Patent No. 4,935,349. Methods for transforming *Acremonium chrysogenum* are disclosed by Sumino *et al.*, U.S. Patent No. 5,162,228. Methods for transforming *Neurospora* are disclosed by Lambowitz, U.S. Patent No. 4,486,533.

For example, the use of *Pichia methanolica* as host for the production of recombinant proteins is disclosed by Raymond, U.S. Patent No. 5,716,808, Raymond, U.S. Patent No. 5,736,383, Raymond *et al.*, *Yeast* 14:11-23 (1998), and in international publication Nos. WO 97/17450, WO 97/17451, WO 98/02536, and WO 98/02565. DNA molecules for use in transforming *P. methanolica* will commonly be prepared as double-stranded, circular plasmids, which are preferably linearized prior to transformation. For polypeptide production in *P. methanolica*, the promoter and terminator in the plasmid can be that of a *P. methanolica* gene, such as a *P. methanolica* alcohol utilization gene (*AUG1* or *AUG2*). Other useful promoters include those of the dihydroxyacetone synthase (DHAS), formate dehydrogenase (FMD), and catalase (CAT) genes. To facilitate integration of the DNA into the host chromosome, it is preferred to have the entire expression segment of the plasmid flanked at both ends by host DNA sequences. A suitable selectable marker for use in *Pichia methanolica* is a *P. methanolica* *ADE2* gene, which encodes phosphoribosyl-5-aminoimidazole carboxylase (AIRC; EC 4.1.1.21), and which allows *ade2* host cells to grow in the absence of adenine. For large-scale, industrial processes where it is desirable to minimize the use of methanol, host cells can be used in which both methanol utilization genes (*AUG1* and *AUG2*) are deleted. For production of secreted proteins, host cells can be deficient in vacuolar protease genes (*PEP4* and *PRB1*). Electroporation is used to facilitate the introduction of a plasmid containing DNA encoding a polypeptide of interest into *P. methanolica* cells. *P. methanolica* cells can be transformed by electroporation using an exponentially decaying, pulsed electric field having a field strength of from 2.5 to 4.5 kV/cm, preferably about 3.75 kV/cm, and a time constant (t) of from 1 to 40 milliseconds, most preferably about 20 milliseconds.



Expression vectors can also be introduced into plant protoplasts, intact plant tissues, or isolated plant cells. Methods for introducing expression vectors into plant tissue include the direct infection or co-cultivation of plant tissue with *Agrobacterium tumefaciens*, microprojectile-mediated delivery, DNA injection, electroporation, and the like. See, for example, Horsch *et al.*, *Science* 227:1229 (1985), Klein *et al.*, *Biotechnology* 10:268 (1992), and Miki *et al.*, "Procedures for Introducing Foreign DNA into Plants," in *Methods in Plant Molecular Biology and Biotechnology*, Glick *et al.* (eds.), pages 67-88 (CRC Press, 1993).

Alternatively, *Zcytor21* genes can be expressed in prokaryotic host cells. Suitable promoters that can be used to express *Zcytor21* polypeptides in a prokaryotic host are well-known to those of skill in the art and include promoters capable of recognizing the T4, T3, Sp6 and T7 polymerases, the P<sub>R</sub> and P<sub>L</sub> promoters of bacteriophage lambda, the *trp*, *recA*, heat shock, *lacUV5*, *tac*, *lpp-lacSpr*, *phoA*, and *lacZ* promoters of *E. coli*, promoters of *B. subtilis*, the promoters of the bacteriophages of *Bacillus*, *Streptomyces* promoters, the *int* promoter of bacteriophage lambda, the *bla* promoter of pBR322, and the CAT promoter of the chloramphenicol acetyl transferase gene. Prokaryotic promoters have been reviewed by Glick, *J. Ind. Microbiol.* 1:277 (1987), Watson *et al.*, *Molecular Biology of the Gene*, 4th Ed. (Benjamin Cummins 1987), and by Ausubel *et al.* (1995).

Suitable prokaryotic hosts include *E. coli* and *Bacillus subtilis*. Suitable strains of *E. coli* include BL21(DE3), BL21(DE3)pLysS, BL21(DE3)pLysE, DH1, DH4I, DH5, DH5I, DH5IF', DH5IMCR, DH10B, DH10B/p3, DH11S, C600, HB101, JM101, JM105, JM109, JM110, K38, RR1, Y1088, Y1089, CSH18, ER1451, and ER1647 (see, for example, Brown (ed.), *Molecular Biology Labfax* (Academic Press 1991)). Suitable strains of *Bacillus subtilis* include BR151, YB886, MI119, MI120, and B170 (see, for example, Hardy, "Bacillus Cloning Methods," in *DNA Cloning: A Practical Approach*, Glover (ed.) (IRL Press 1985)).

When expressing a *Zcytor21* polypeptide in bacteria such as *E. coli*, the polypeptide may be retained in the cytoplasm, typically as insoluble granules, or may be directed to the periplasmic space by a bacterial secretion sequence. In the former case, the cells are lysed, and the granules are recovered and denatured using, for example,

guanidine isothiocyanate or urea. The denatured polypeptide can then be refolded and dimerized by diluting the denaturant, such as by dialysis against a solution of urea and a combination of reduced and oxidized glutathione, followed by dialysis against a buffered saline solution. In the latter case, the polypeptide can be recovered from the periplasmic space in a soluble and functional form by disrupting the cells (by, for example, sonication or osmotic shock) to release the contents of the periplasmic space and recovering the protein, thereby obviating the need for denaturation and refolding.

Methods for expressing proteins in prokaryotic hosts are well-known to those of skill in the art (see, for example, Williams *et al.*, "Expression of foreign proteins in *E. coli* using plasmid vectors and purification of specific polyclonal antibodies," in *DNA Cloning 2: Expression Systems, 2nd Edition*, Glover *et al.* (eds.), page 15 (Oxford University Press 1995), Ward *et al.*, "Genetic Manipulation and Expression of Antibodies," in *Monoclonal Antibodies: Principles and Applications*, page 137 (Wiley-Liss, Inc. 1995), and Georgiou, "Expression of Proteins in Bacteria," in *Protein Engineering: Principles and Practice*, Cleland *et al.* (eds.), page 101 (John Wiley & Sons, Inc. 1996)).

Standard methods for introducing expression vectors into bacterial, yeast, insect, and plant cells are provided, for example, by Ausubel (1995).

General methods for expressing and recovering foreign protein produced by a mammalian cell system are provided by, for example, Etcheverry, "Expression of Engineered Proteins in Mammalian Cell Culture," in *Protein Engineering: Principles and Practice*, Cleland *et al.* (eds.), pages 163 (Wiley-Liss, Inc. 1996). Standard techniques for recovering protein produced by a bacterial system is provided by, for example, Grişshammer *et al.*, "Purification of over-produced proteins from *E. coli* cells," in *DNA Cloning 2: Expression Systems, 2nd Edition*, Glover *et al.* (eds.), pages 59-92 (Oxford University Press 1995). Established methods for isolating recombinant proteins from a baculovirus system are described by Richardson (ed.), *Baculovirus Expression Protocols* (The Humana Press, Inc. 1995).

As an alternative, polypeptides of the present invention can be synthesized by exclusive solid phase synthesis, partial solid phase methods, fragment condensation or classical solution synthesis. These synthesis methods are well-known

to those of skill in the art (see, for example, Merrifield, *J. Am. Chem. Soc.* 85:2149 (1963), Stewart *et al.*, "Solid Phase Peptide Synthesis" (2nd Edition), (Pierce Chemical Co. 1984), Bayer and Rapp, *Chem. Pept. Prot.* 3:3 (1986), Atherton *et al.*, *Solid Phase Peptide Synthesis: A Practical Approach* (IRL Press 1989), Fields and Colowick, 5 "Solid-Phase Peptide Synthesis," *Methods in Enzymology Volume 289* (Academic Press 1997), and Lloyd-Williams *et al.*, *Chemical Approaches to the Synthesis of Peptides and Proteins* (CRC Press, Inc. 1997)). Variations in total chemical synthesis strategies, such as "native chemical ligation" and "expressed protein ligation" are also standard (see, for example, Dawson *et al.*, *Science* 266:776 (1994), Hackeng *et al.*, *Proc. Nat'l Acad. Sci. USA* 94:7845 (1997), Dawson, *Methods Enzymol.* 287: 34 (1997), Muir *et al.*, 10 *Proc. Nat'l Acad. Sci. USA* 95:6705 (1998), and Severinov and Muir, *J. Biol. Chem.* 273:16205 (1998)).

Peptides and polypeptides of the present invention comprise at least six, at least nine, or at least 15 contiguous amino acid residues of SEQ ID NO:2, SEQ ID 15 NO:5, SEQ ID NO:8, SEQ ID NO:11, SEQ ID NO:15. As an illustration, polypeptides can comprise at least six, at least nine, or at least 15 contiguous amino acid residues of any of the following amino acid sequences: (a) amino acid residues 24 to 667 of SEQ ID NO:11, (b) amino acid residues 24 to 589 of SEQ ID NO:2, (c) amino acid residues 24 to 609 of SEQ ID NO:5, (d) amino acid residues 24 to 533 of SEQ ID NO:8, (e) 20 amino acid residues 24 to 657 of SEQ ID NO:15, (f) amino acid residues 24 to 454 of SEQ ID NO:11, (g) amino acid residues 24 to 376 of SEQ ID NO:2, (h) amino acid residues 24 to 396 of SEQ ID NO:5, or (i) amino acid residues 24 to 444 of SEQ ID NO:15. Within certain embodiments of the invention, the polypeptides comprise 20, 30, 40, 50, 100, or more contiguous residues of these amino acid sequences. For 25 example, polypeptides can comprise at least 30 contiguous amino acid residues of an amino acid sequence selected from the group consisting of: (a) amino acid residues 24 to 667 of SEQ ID NO:11, (b) amino acid residues 24 to 589 of SEQ ID NO:2, (c) amino acid residues 24 to 609 of SEQ ID NO:5, (d) amino acid residues 24 to 533 of SEQ ID NO:8, (e) amino acid residues 24 to 657 of SEQ ID NO:15, (f) amino acid residues 24 30 to 454 of SEQ ID NO:11, (g) amino acid residues 24 to 376 of SEQ ID NO:2, (h) amino acid residues 24 to 396 of SEQ ID NO:5, or (i) amino acid residues 24 to 444 of

SEQ ID NO:15. Nucleic acid molecules encoding such peptides and polypeptides are useful as polymerase chain reaction primers and probes.

#### 6. *Production of Zcytor21 Fusion Proteins and Conjugates*

5 One general class of Zcytor21 analogs are variants having an amino acid sequence that is a mutation of the amino acid sequence disclosed herein. Another general class of Zcytor21 analogs is provided by anti-idiotypic antibodies, and fragments thereof, as described below. Moreover, recombinant antibodies comprising anti-idiotypic variable domains can be used as analogs (see, for example, Monfardini *et al.*,  
10 *Proc. Assoc. Am. Physicians* 108:420 (1996)). Since the variable domains of anti-idiotypic Zcytor21 antibodies mimic Zcytor21, these domains can provide Zcytor21 binding activity. Methods of producing anti-idiotypic catalytic antibodies are known to those of skill in the art (see, for example, Joron *et al.*, *Ann. N Y Acad. Sci.* 672:216 (1992), Friboulet *et al.*, *Appl. Biochem. Biotechnol.* 47:229 (1994), and Avasle *et al.*,  
15 *Ann. N Y Acad. Sci.* 864:118 (1998)).

Another approach to identifying Zcytor21 analogs is provided by the use of combinatorial libraries. Methods for constructing and screening phage display and other combinatorial libraries are provided, for example, by Kay *et al.*, *Phage Display of Peptides and Proteins* (Academic Press 1996), Verdine, U.S. Patent No. 5,783,384,  
20 Kay, *et al.*, U.S. Patent No. 5,747,334, and Kauffman *et al.*, U.S. Patent No. 5,723,323.

Zcytor21 polypeptides have both *in vivo* and *in vitro* uses. As an illustration, a soluble form of Zcytor21 can be added to cell culture medium to inhibit the effects of the Zcytor21 ligand produced by the cultured cells.

Fusion proteins of Zcytor21 can be used to express Zcytor21 in a  
25 recombinant host, and to isolate the produced Zcytor21. As described below, particular Zcytor21 fusion proteins also have uses in diagnosis and therapy. One type of fusion protein comprises a peptide that guides a Zcytor21 polypeptide from a recombinant host cell. To direct a Zcytor21 polypeptide into the secretory pathway of a eukaryotic host cell, a secretory signal sequence (also known as a signal peptide, a leader sequence, prepro sequence or pre sequence) is provided in the Zcytor21 expression vector. While  
30 the secretory signal sequence may be derived from Zcytor21, a suitable signal sequence

may also be derived from another secreted protein or synthesized *de novo*. The secretory signal sequence is operably linked to a *Zcytor21*-encoding sequence such that the two sequences are joined in the correct reading frame and positioned to direct the newly synthesized polypeptide into the secretory pathway of the host cell. Secretory  
5 signal sequences are commonly positioned 5' to the nucleotide sequence encoding the polypeptide of interest, although certain secretory signal sequences may be positioned elsewhere in the nucleotide sequence of interest (see, *e.g.*, Welch *et al.*, U.S. Patent No. 5,037,743; Holland *et al.*, U.S. Patent No. 5,143,830).

Although the secretory signal sequence of *Zcytor21* or another protein  
10 produced by mammalian cells (*e.g.*, tissue-type plasminogen activator signal sequence, as described, for example, in U.S. Patent No. 5,641,655) is useful for expression of *Zcytor21* in recombinant mammalian hosts, a yeast signal sequence is preferred for expression in yeast cells. Examples of suitable yeast signal sequences are those derived from yeast mating pheromone  $\alpha$ -factor (encoded by the *MF $\alpha$ 1* gene), invertase (encoded  
15 by the *SUC2* gene), or acid phosphatase (encoded by the *PHO5* gene). See, for example, Romanos *et al.*, "Expression of Cloned Genes in Yeast," in *DNA Cloning 2: A Practical Approach*, 2<sup>nd</sup> Edition, Glover and Hames (eds.), pages 123-167 (Oxford University Press 1995).

In bacterial cells, it is often desirable to express a heterologous protein  
20 as a fusion protein to decrease toxicity, increase stability, and to enhance recovery of the expressed protein. For example, *Zcytor21* can be expressed as a fusion protein comprising a glutathione S-transferase polypeptide. Glutathione S-transferase fusion proteins are typically soluble, and easily purifiable from *E. coli* lysates on immobilized glutathione columns. In similar approaches, a *Zcytor21* fusion protein comprising a  
25 maltose binding protein polypeptide can be isolated with an amylose resin column, while a fusion protein comprising the C-terminal end of a truncated Protein A gene can be purified using IgG-Sepharose. Established techniques for expressing a heterologous polypeptide as a fusion protein in a bacterial cell are described, for example, by Williams *et al.*, "Expression of Foreign Proteins in *E. coli* Using Plasmid Vectors and  
30 Purification of Specific Polyclonal Antibodies," in *DNA Cloning 2: A Practical Approach*, 2<sup>nd</sup> Edition, Glover and Hames (Eds.), pages 15-58 (Oxford University Press

1995). In addition, commercially available expression systems are available. For example, the PINPOINT Xa protein purification system (Promega Corporation; Madison, WI) provides a method for isolating a fusion protein comprising a polypeptide that becomes biotinylated during expression with a resin that comprises avidin.

5           Peptide tags that are useful for isolating heterologous polypeptides expressed by either prokaryotic or eukaryotic cells include polyHistidine tags (which have an affinity for nickel-chelating resin), *c-myc* tags, calmodulin binding protein (isolated with calmodulin affinity chromatography), substance P, the RYIRS tag (which binds with anti-RYIRS antibodies), the Glu-Glu tag, and the FLAG tag (which binds  
10 with anti-FLAG antibodies). See, for example, Luo *et al.*, *Arch. Biochem. Biophys.* 329:215 (1996), Morganti *et al.*, *Biotechnol. Appl. Biochem.* 23:67 (1996), and Zheng *et al.*, *Gene* 186:55 (1997). Nucleic acid molecules encoding such peptide tags are available, for example, from Sigma-Aldrich Corporation (St. Louis, MO).

          The present invention also contemplates that the use of the secretory  
15 signal sequence contained in the Zcytor21 polypeptides of the present invention to direct other polypeptides into the secretory pathway. A signal fusion polypeptide can be made wherein a secretory signal sequence derived from, for example, amino acid residues 1 to 23 of SEQ ID NO:2 is operably linked to another polypeptide using methods known in the art and disclosed herein. The secretory signal sequence  
20 contained in the fusion polypeptides of the present invention is preferably fused amino-terminally to an additional peptide to direct the additional peptide into the secretory pathway. Such constructs have numerous applications known in the art. For example, these novel secretory signal sequence fusion constructs can direct the secretion of an active component of a normally non-secreted protein, such as a receptor. Such fusions  
25 may be used in a transgenic animal or in a cultured recombinant host to direct peptides through the secretory pathway. With regard to the latter, exemplary polypeptides include pharmaceutically active molecules such as Factor VIIa, proinsulin, insulin, follicle stimulating hormone, tissue type plasminogen activator, tumor necrosis factor, interleukins (*e.g.*, interleukin-1 (IL-1), IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-  
30 10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, and IL-21), colony stimulating factors (*e.g.*, granulocyte-colony stimulating factor (G-CSF) and

granulocyte macrophage-colony stimulating factor (GM-CSF)), interferons (*e.g.*, interferons- $\alpha$ , - $\beta$ , - $\gamma$ , - $\omega$ , - $\delta$ , - $\epsilon$ , and - $\tau$ ), the stem cell growth factor designated "S1 factor," erythropoietin, and thrombopoietin. The Zcytor21 secretory signal sequence contained in the fusion polypeptides of the present invention is preferably fused amino-terminally to an additional peptide to direct the additional peptide into the secretory pathway. Fusion proteins comprising a Zcytor21 secretory signal sequence can be constructed using standard techniques.

Another form of fusion protein comprises a Zcytor21 polypeptide and an immunoglobulin heavy chain constant region, typically an F<sub>C</sub> fragment, which contains two or three constant region domains and a hinge region but lacks the variable region. As an illustration, Chang *et al.*, U.S. Patent No. 5,723,125, describe a fusion protein comprising a human interferon and a human immunoglobulin Fc fragment. The C-terminal of the interferon is linked to the N-terminal of the Fc fragment by a peptide linker moiety. An example of a peptide linker is a peptide comprising primarily a T cell inert sequence, which is immunologically inert. An exemplary peptide linker has the amino acid sequence: GGS GG SGGGG SGGGG S (SEQ ID NO:13). In this fusion protein, an illustrative Fc moiety is a human  $\gamma$ 4 chain, which is stable in solution and has little or no complement activating activity. Accordingly, the present invention contemplates a Zcytor21 fusion protein that comprises a Zcytor21 moiety and a human Fc fragment, wherein the C-terminus of the Zcytor21 moiety is attached to the N-terminus of the Fc fragment via a peptide linker, such as a peptide consisting of the amino acid sequence of SEQ ID NO:13. The Zcytor21 moiety can be a Zcytor21 molecule or a fragment thereof. For example, a fusion protein can comprise an Fc fragment (*e.g.*, a human Fc fragment), and amino acid residues 24 to 454 of SEQ ID NO:11, amino acid residues 24 to 376 of SEQ ID NO:2, amino acid residues 24 to 396 of SEQ ID NO:5, amino acid residues 24 to 533 of SEQ ID NO:8, or amino acid residues 24 to 444 of SEQ ID NO:15.

In another variation, a Zcytor21 fusion protein comprises an IgG sequence, a Zcytor21 moiety covalently joined to the amino terminal end of the IgG sequence, and a signal peptide that is covalently joined to the amino terminal of the Zcytor21 moiety, wherein the IgG sequence consists of the following elements in the

following order: a hinge region, a CH<sub>2</sub> domain, and a CH<sub>3</sub> domain. Accordingly, the IgG sequence lacks a CH<sub>1</sub> domain. The Zcytor21 moiety displays a Zcytor21 activity, as described herein, such as the ability to bind with a Zcytor21 ligand. This general approach to producing fusion proteins that comprise both antibody and nonantibody portions has been described by LaRochelle *et al.*, EP 742830 (WO 95/21258).

Fusion proteins comprising a Zcytor21 moiety and an Fc moiety can be used, for example, as an *in vitro* assay tool. For example, the presence of a Zcytor21 ligand in a biological sample can be detected using a Zcytor21-immunoglobulin fusion protein, in which the Zcytor21 moiety is used to bind the ligand, and a macromolecule, such as Protein A or anti-Fc antibody, is used to bind the fusion protein to a solid support. Such systems can be used to identify agonists and antagonists that interfere with the binding of a Zcytor21 ligand to its receptor.

Other examples of antibody fusion proteins include polypeptides that comprise an antigen-binding domain and a Zcytor21 fragment that contains a Zcytor21 extracellular domain. Such molecules can be used to target particular tissues for the benefit of Zcytor21 binding activity.

The present invention further provides a variety of other polypeptide fusions. For example, part or all of a domain(s) conferring a biological function can be swapped between Zcytor21 of the present invention with the functionally equivalent domain(s) from another member of the cytokine receptor family. Polypeptide fusions can be expressed in recombinant host cells to produce a variety of Zcytor21 fusion analogs. A Zcytor21 polypeptide can be fused to two or more moieties or domains, such as an affinity tag for purification and a targeting domain. Polypeptide fusions can also comprise one or more cleavage sites, particularly between domains. See, for example, Tuan *et al.*, *Connective Tissue Research* 34:1 (1996).

Fusion proteins can be prepared by methods known to those skilled in the art by preparing each component of the fusion protein and chemically conjugating them. Alternatively, a polynucleotide encoding both components of the fusion protein in the proper reading frame can be generated using known techniques and expressed by the methods described herein. General methods for enzymatic and chemical cleavage



of fusion proteins are described, for example, by Ausubel (1995) at pages 16-19 to 16-25.

Zcytor21 polypeptides can be used to identify and to isolate Zcytor21 ligands. For example, proteins and peptides of the present invention can be  
5 immobilized on a column and used to bind ligands from a biological sample that is run over the column (Hermanson *et al.* (eds.), *Immobilized Affinity Ligand Techniques*, pages 195-202 (Academic Press 1992)).

The activity of a Zcytor21 polypeptide can be observed by a silicon-based biosensor microphysiometer, which measures the extracellular acidification rate  
10 or proton excretion associated with receptor binding and subsequent physiologic cellular responses. An exemplary device is the CYTOSENSOR Microphysiometer manufactured by Molecular Devices, Sunnyvale, CA. A variety of cellular responses, such as cell proliferation, ion transport, energy production, inflammatory response, regulatory and receptor activation, and the like, can be measured by this method (see,  
15 for example, McConnell *et al.*, *Science* 257:1906 (1992), Pitchford *et al.*, *Meth. Enzymol.* 228:84 (1997), Arimilli *et al.*, *J. Immunol. Meth.* 212:49 (1998), Van Liefde *et al.*, *Eur. J. Pharmacol.* 346:87 (1998)). The microphysiometer can be used for assaying eukaryotic, prokaryotic, adherent, or non-adherent cells. By measuring extracellular acidification changes in cell media over time, the microphysiometer  
20 directly measures cellular responses to various stimuli, including agonists, ligands, or antagonists of Zcytor21.

For example, the microphysiometer is used to measure responses of an Zcytor21-expressing eukaryotic cell, compared to a control eukaryotic cell that does not express Zcytor21 polypeptide. Suitable cells responsive to Zcytor21-modulating  
25 stimuli include recombinant host cells comprising a Zcytor21 expression vector, and cells that naturally express Zcytor21. Extracellular acidification provides one measure for a Zcytor21-modulated cellular response. In addition, this approach can be used to identify ligands, agonists, and antagonists of Zcytor21 ligand. For example, a molecule can be identified as an agonist of Zcytor21 ligand by providing cells that express a  
30 Zcytor21 polypeptide, culturing a first portion of the cells in the absence of the test compound, culturing a second portion of the cells in the presence of the test compound,

and determining whether the second portion exhibits a cellular response, in comparison with the first portion.

Alternatively, a solid phase system can be used to identify a Zcytor21 ligand, or an agonist or antagonist of a Zcytor21 ligand. For example, a Zcytor21 polypeptide or Zcytor21 fusion protein can be immobilized onto the surface of a receptor chip of a commercially available biosensor instrument (BIAcore, Biacore AB; Uppsala, Sweden). The use of this instrument is disclosed, for example, by Karlsson, *Immunol. Methods* 145:229 (1991), and Cunningham and Wells, *J. Mol. Biol.* 234:554 (1993).

In brief, a Zcytor21 polypeptide or fusion protein is covalently attached, using amine or sulfhydryl chemistry, to dextran fibers that are attached to gold film within a flow cell. A test sample is then passed through the cell. If a ligand is present in the sample, it will bind to the immobilized polypeptide or fusion protein, causing a change in the refractive index of the medium, which is detected as a change in surface plasmon resonance of the gold film. This system allows the determination of on- and off-rates, from which binding affinity can be calculated, and assessment of stoichiometry of binding. This system can also be used to examine antibody-antigen interactions, and the interactions of other complement/anti-complement pairs.

Zcytor21 binding domains can be further characterized by physical analysis of structure, as determined by such techniques as nuclear magnetic resonance, crystallography, electron diffraction or photoaffinity labeling, in conjunction with mutation of putative contact site amino acids of Zcytor21 ligand agonists. See, for example, de Vos *et al.*, *Science* 255:306 (1992), Smith *et al.*, *J. Mol. Biol.* 224:899 (1992), and Wlodaver *et al.*, *FEBS Lett.* 309:59 (1992).

The present invention also contemplates chemically modified Zcytor21 compositions, in which a Zcytor21 polypeptide is linked with a polymer. Illustrative Zcytor21 polypeptides are soluble polypeptides that lack a functional transmembrane domain, such as a polypeptide consisting of amino acid residues 24 to 454 of SEQ ID NO:11, amino acid residues 24 to 376 of SEQ ID NO:2, amino acid residues 24 to 396 of SEQ ID NO:5, amino acid residues 24 to 533 of SEQ ID NO:8, or amino acid residues 24 to 444 of SEQ ID NO:15. Typically, the polymer is water-soluble so that

the Zcytor21 conjugate does not precipitate in an aqueous environment, such as a physiological environment. An example of a suitable polymer is one that has been modified to have a single reactive group, such as an active ester for acylation, or an aldehyde for alkylation. In this way, the degree of polymerization can be controlled.

5 An example of a reactive aldehyde is polyethylene glycol propionaldehyde, or mono-(C<sub>1</sub>-C<sub>10</sub>) alkoxy, or aryloxy derivatives thereof (see, for example, Harris, *et al.*, U.S. Patent No. 5,252,714). The polymer may be branched or unbranched. Moreover, a mixture of polymers can be used to produce Zcytor21 conjugates.

Zcytor21 conjugates used for therapy can comprise pharmaceutically acceptable water-soluble polymer moieties. Suitable water-soluble polymers include  
10 polyethylene glycol (PEG), monomethoxy-PEG, mono-(C<sub>1</sub>-C<sub>10</sub>)alkoxy-PEG, aryloxy-PEG, poly-(N-vinyl pyrrolidone)PEG, tresyl monomethoxy PEG, PEG propionaldehyde, *bis*-succinimidyl carbonate PEG, propylene glycol homopolymers, a polypropylene oxide/ethylene oxide co-polymer, polyoxyethylated polyols (*e.g.*,  
15 glycerol), polyvinyl alcohol, dextran, cellulose, or other carbohydrate-based polymers. Suitable PEG may have a molecular weight from about 600 to about 60,000, including, for example, 5,000, 12,000, 20,000 and 25,000. A Zcytor21 conjugate can also comprise a mixture of such water-soluble polymers.

One example of a Zcytor21 conjugate comprises a Zcytor21 moiety and  
20 a polyalkyl oxide moiety attached to the *N*-terminus of the Zcytor21 moiety. PEG is one suitable polyalkyl oxide. As an illustration, Zcytor21 can be modified with PEG, a process known as "PEGylation." PEGylation of Zcytor21 can be carried out by any of the PEGylation reactions known in the art (see, for example, EP 0 154 316, Delgado *et al.*, *Critical Reviews in Therapeutic Drug Carrier Systems* 9:249 (1992), Duncan and Spreafico, *Clin. Pharmacokinet.* 27:290 (1994), and Francis *et al.*, *Int J Hematol* 68:1  
25 (1998)). For example, PEGylation can be performed by an acylation reaction or by an alkylation reaction with a reactive polyethylene glycol molecule. In an alternative approach, Zcytor21 conjugates are formed by condensing activated PEG, in which a terminal hydroxy or amino group of PEG has been replaced by an activated linker (see,  
30 for example, Karasiewicz *et al.*, U.S. Patent No. 5,382,657).

PEGylation by acylation typically requires reacting an active ester derivative of PEG with a Zcytor21 polypeptide. An example of an activated PEG ester is PEG esterified to *N*-hydroxysuccinimide. As used herein, the term "acylation" includes the following types of linkages between Zcytor21 and a water-soluble polymer: amide, carbamate, urethane, and the like. Methods for preparing PEGylated Zcytor21 by acylation will typically comprise the steps of (a) reacting a Zcytor21 polypeptide with PEG (such as a reactive ester of an aldehyde derivative of PEG) under conditions whereby one or more PEG groups attach to Zcytor21, and (b) obtaining the reaction product(s). Generally, the optimal reaction conditions for acylation reactions will be determined based upon known parameters and desired results. For example, the larger the ratio of PEG:Zcytor21, the greater the percentage of polyPEGylated Zcytor21 product.

The product of PEGylation by acylation is typically a polyPEGylated Zcytor21 product, wherein the lysine  $\epsilon$ -amino groups are PEGylated via an acyl linking group. An example of a connecting linkage is an amide. Typically, the resulting Zcytor21 will be at least 95% mono-, di-, or tri-pegylated, although some species with higher degrees of PEGylation may be formed depending upon the reaction conditions. PEGylated species can be separated from unconjugated Zcytor21 polypeptides using standard purification methods, such as dialysis, ultrafiltration, ion exchange chromatography, affinity chromatography, and the like.

PEGylation by alkylation generally involves reacting a terminal aldehyde derivative of PEG with Zcytor21 in the presence of a reducing agent. PEG groups can be attached to the polypeptide via a  $-\text{CH}_2\text{-NH}$  group.

Derivatization via reductive alkylation to produce a monoPEGylated product takes advantage of the differential reactivity of different types of primary amino groups available for derivatization. Typically, the reaction is performed at a pH that allows one to take advantage of the pKa differences between the  $\epsilon$ -amino groups of the lysine residues and the  $\alpha$ -amino group of the *N*-terminal residue of the protein. By such selective derivatization, attachment of a water-soluble polymer that contains a reactive group such as an aldehyde, to a protein is controlled. The conjugation with the polymer occurs predominantly at the *N*-terminus of the protein without significant modification

of other reactive groups such as the lysine side chain amino groups. The present invention provides a substantially homogenous preparation of Zcytor21 monopolymer conjugates.

Reductive alkylation to produce a substantially homogenous population of monopolymer Zcytor21 conjugate molecule can comprise the steps of: (a) reacting a  
5 of monopolymer Zcytor21 conjugate molecule can comprise the steps of: (a) reacting a Zcytor21 polypeptide with a reactive PEG under reductive alkylation conditions at a pH suitable to permit selective modification of the  $\alpha$ -amino group at the amino terminus of the Zcytor21, and (b) obtaining the reaction product(s). The reducing agent used for reductive alkylation should be stable in aqueous solution and able to reduce only the  
10 Schiff base formed in the initial process of reductive alkylation. Illustrative reducing agents include sodium borohydride, sodium cyanoborohydride, dimethylamine borane, trimethylamine borane, and pyridine borane.

For a substantially homogenous population of monopolymer Zcytor21 conjugates, the reductive alkylation reaction conditions are those that permit the  
15 selective attachment of the water soluble polymer moiety to the *N*-terminus of Zcytor21. Such reaction conditions generally provide for pKa differences between the lysine amino groups and the  $\alpha$ -amino group at the *N*-terminus. The pH also affects the ratio of polymer to protein to be used. In general, if the pH is lower, a larger excess of polymer to protein will be desired because the less reactive the *N*-terminal  $\alpha$ -group, the  
20 more polymer is needed to achieve optimal conditions. If the pH is higher, the polymer:Zcytor21 need not be as large because more reactive groups are available. Typically, the pH will fall within the range of 3 to 9, or 3 to 6.

Another factor to consider is the molecular weight of the water-soluble polymer. Generally, the higher the molecular weight of the polymer, the fewer number  
25 of polymer molecules which may be attached to the protein. For PEGylation reactions, the typical molecular weight is about 2 kDa to about 100 kDa, about 5 kDa to about 50 kDa, or about 12 kDa to about 25 kDa. The molar ratio of water-soluble polymer to Zcytor21 will generally be in the range of 1:1 to 100:1. Typically, the molar ratio of water-soluble polymer to Zcytor21 will be 1:1 to 20:1 for polyPEGylation, and 1:1 to  
30 5:1 for monoPEGylation.

General methods for producing conjugates comprising a polypeptide and water-soluble polymer moieties are known in the art. See, for example, Karasiewicz *et al.*, U.S. Patent No. 5,382,657, Greenwald *et al.*, U.S. Patent No. 5,738, 846, Nieforth *et al.*, *Clin. Pharmacol. Ther.* 59:636 (1996), Monkarsch *et al.*, *Anal. Biochem.* 247:434 (1997)).

The present invention contemplates compositions comprising a peptide or polypeptide described herein. Such compositions can further comprise a carrier. The carrier can be a conventional organic or inorganic carrier. Examples of carriers include water, buffer solution, alcohol, propylene glycol, macrogol, sesame oil, corn oil, and the like.

#### 7. Isolation of Zcytor21 Polypeptides

The polypeptides of the present invention can be purified to at least about 80% purity, to at least about 90% purity, to at least about 95% purity, or greater than 95% purity with respect to contaminating macromolecules, particularly other proteins and nucleic acids, and free of infectious and pyrogenic agents. The polypeptides of the present invention may also be purified to a pharmaceutically pure state, which is greater than 99.9% pure. In certain preparations, purified polypeptide is substantially free of other polypeptides, particularly other polypeptides of animal origin.

Fractionation and/or conventional purification methods can be used to obtain preparations of Zcytor21 purified from natural sources (*e.g.*, skin tissue), synthetic Zcytor21 polypeptides, and recombinant Zcytor21 polypeptides and fusion Zcytor21 polypeptides purified from recombinant host cells. In general, ammonium sulfate precipitation and acid or chaotrope extraction may be used for fractionation of samples. Exemplary purification steps may include hydroxyapatite, size exclusion, FPLC and reverse-phase high performance liquid chromatography. Suitable chromatographic media include derivatized dextrans, agarose, cellulose, polyacrylamide, specialty silicas, and the like. PEI, DEAE, QAE and Q derivatives are suitable. Exemplary chromatographic media include those media derivatized with phenyl, butyl, or octyl groups, such as Phenyl-Sepharose FF (Pharmacia), Toyopearl butyl 650 (Toso Haas, Montgomeryville, PA), Octyl-Sepharose (Pharmacia) and the

like; or polyacrylic resins, such as Amberchrom CG 71 (Toso Haas) and the like. Suitable solid supports include glass beads, silica-based resins, cellulosic resins, agarose beads, cross-linked agarose beads, polystyrene beads, cross-linked polyacrylamide resins and the like that are insoluble under the conditions in which they are to be used. These supports may be modified with reactive groups that allow attachment of proteins by amino groups, carboxyl groups, sulfhydryl groups, hydroxyl groups and/or carbohydrate moieties.

Examples of coupling chemistries include cyanogen bromide activation, N-hydroxysuccinimide activation, epoxide activation, sulfhydryl activation, hydrazide activation, and carboxyl and amino derivatives for carbodiimide coupling chemistries. These and other solid media are well known and widely used in the art, and are available from commercial suppliers. Selection of a particular method for polypeptide isolation and purification is a matter of routine design and is determined in part by the properties of the chosen support. See, for example, *Affinity Chromatography: Principles & Methods* (Pharmacia LKB Biotechnology 1988), and Doonan, *Protein Purification Protocols* (The Humana Press 1996).

Additional variations in Zcytor21 isolation and purification can be devised by those of skill in the art. For example, anti-Zcytor21 antibodies, obtained as described below, can be used to isolate large quantities of protein by immunoaffinity purification.

The polypeptides of the present invention can also be isolated by exploitation of particular properties. For example, immobilized metal ion adsorption (IMAC) chromatography can be used to purify histidine-rich proteins, including those comprising polyhistidine tags. Briefly, a gel is first charged with divalent metal ions to form a chelate (Sulkowski, *Trends in Biochem. Sci.* 3:1 (1985)). Histidine-rich proteins will be adsorbed to this matrix with differing affinities, depending upon the metal ion used, and will be eluted by competitive elution, lowering the pH, or use of strong chelating agents. Other methods of purification include purification of glycosylated proteins by lectin affinity chromatography and ion exchange chromatography (M. Deutscher, (ed.), *Meth. Enzymol.* 182:529 (1990)). Within additional embodiments of the invention, a

fusion of the polypeptide of interest and an affinity tag (*e.g.*, maltose-binding protein, an immunoglobulin domain) may be constructed to facilitate purification.

Zcytor21 polypeptides or fragments thereof may also be prepared through chemical synthesis, as described above. Zcytor21 polypeptides may be monomers or multimers; glycosylated or non-glycosylated; PEGylated or non-PEGylated; and may or may not include an initial methionine amino acid residue.

#### 8. *Production of Antibodies to Zcytor21 Proteins*

Antibodies to Zcytor21 can be obtained, for example, using the product of a Zcytor21 expression vector or Zcytor21 isolated from a natural source as an antigen. Particularly useful anti-Zcytor21 antibodies "bind specifically" with Zcytor21. Antibodies are considered to be specifically binding if the antibodies exhibit at least one of the following two properties: (1) antibodies bind to Zcytor21 with a threshold level of binding activity, and (2) antibodies do not significantly cross-react with polypeptides related to Zcytor21.

With regard to the first characteristic, antibodies specifically bind if they bind to a Zcytor21 polypeptide, peptide or epitope with a binding affinity ( $K_a$ ) of  $10^6 M^{-1}$  or greater, preferably  $10^7 M^{-1}$  or greater, more preferably  $10^8 M^{-1}$  or greater, and most preferably  $10^9 M^{-1}$  or greater. The binding affinity of an antibody can be readily determined by one of ordinary skill in the art, for example, by Scatchard analysis (Scatchard, *Ann. NY Acad. Sci.* 51:660 (1949)). With regard to the second characteristic, antibodies do not significantly cross-react with related polypeptide molecules, for example, if they detect Zcytor21, but not presently known polypeptides using a standard Western blot analysis. Examples of known related polypeptides include known cytokine receptors.

Anti-Zcytor21 antibodies can be produced using antigenic Zcytor21 epitope-bearing peptides and polypeptides. Antigenic epitope-bearing peptides and polypeptides of the present invention contain a sequence of at least nine, or between 15 to about 30 amino acids contained within SEQ ID NO:2 or another amino acid sequence disclosed herein. However, peptides or polypeptides comprising a larger portion of an amino acid sequence of the invention, containing from 30 to 50 amino



acids, or any length up to and including the entire amino acid sequence of a polypeptide of the invention, also are useful for inducing antibodies that bind with Zcytor21. It is desirable that the amino acid sequence of the epitope-bearing peptide is selected to provide substantial solubility in aqueous solvents (*i.e.*, the sequence includes relatively hydrophilic residues, while hydrophobic residues are typically avoided). Moreover, amino acid sequences containing proline residues may be also be desirable for antibody production.

As an illustration, potential antigenic sites in Zcytor21 were identified using the Jameson-Wolf method, Jameson and Wolf, *CABIOS* 4:181, (1988), as implemented by the PROTEAN program (version 3.14) of LASERGENE (DNASTAR; Madison, WI). Default parameters were used in this analysis.

The Jameson-Wolf method predicts potential antigenic determinants by combining six major subroutines for protein structural prediction. Briefly, the Hopp-Woods method, Hopp *et al.*, *Proc. Nat'l Acad. Sci. USA* 78:3824 (1981), was first used to identify amino acid sequences representing areas of greatest local hydrophilicity (parameter: seven residues averaged). In the second step, Emini's method, Emini *et al.*, *J. Virology* 55:836 (1985), was used to calculate surface probabilities (parameter: surface decision threshold  $(0.6) = 1$ ). Third, the Karplus-Schultz method, Karplus and Schultz, *Naturwissenschaften* 72:212 (1985), was used to predict backbone chain flexibility (parameter: flexibility threshold  $(0.2) = 1$ ). In the fourth and fifth steps of the analysis, secondary structure predictions were applied to the data using the methods of Chou-Fasman, Chou, "Prediction of Protein Structural Classes from Amino Acid Composition," in *Prediction of Protein Structure and the Principles of Protein Conformation*, Fasman (ed.), pages 549-586 (Plenum Press 1990), and Garnier-Robson, Garnier *et al.*, *J. Mol. Biol.* 120:97 (1978) (Chou-Fasman parameters: conformation table = 64 proteins;  $\alpha$  region threshold = 103;  $\beta$  region threshold = 105; Garnier-Robson parameters:  $\alpha$  and  $\beta$  decision constants = 0). In the sixth subroutine, flexibility parameters and hydropathy/solvent accessibility factors were combined to determine a surface contour value, designated as the "antigenic index." Finally, a peak broadening function was applied to the antigenic index, which broadens major surface peaks by adding 20, 40, 60, or 80% of the respective peak value to account for additional free

energy derived from the mobility of surface regions relative to interior regions. This calculation was not applied, however, to any major peak that resides in a helical region, since helical regions tend to be less flexible.

The results of this analysis indicated that the following amino acid sequences of SEQ ID NO:11 would provide suitable antigenic molecules: amino acids 44 to 52, amino acids 101 to 109, amino acids 121 to 136, amino acids 141 to 180, amino acids 250 to 261, amino acids 313 to 328, amino acids 477 to 488, amino acids 562 to 574, amino acids 597 to 622, and amino acids 641 to 652. The present invention contemplates the use of any one of these antigenic amino acid sequences to generate antibodies to Zcytor21-d2. The present invention also contemplates polypeptides comprising at least one of these antigenic molecules. Similar analyses can be performed with the other Zcytor21 amino acid sequences disclosed herein.

Polyclonal antibodies to recombinant Zcytor21 protein or to Zcytor21 isolated from natural sources can be prepared using methods well-known to those of skill in the art. See, for example, Green *et al.*, "Production of Polyclonal Antisera," in *Immunochemical Protocols* (Manson, ed.), pages 1-5 (Humana Press 1992), and Williams *et al.*, "Expression of foreign proteins in *E. coli* using plasmid vectors and purification of specific polyclonal antibodies," in *DNA Cloning 2: Expression Systems, 2nd Edition*, Glover *et al.* (eds.), page 15 (Oxford University Press 1995). The immunogenicity of a Zcytor21 polypeptide can be increased through the use of an adjuvant, such as alum (aluminum hydroxide) or Freund's complete or incomplete adjuvant. Polypeptides useful for immunization also include fusion polypeptides, such as fusions of Zcytor21 or a portion thereof with an immunoglobulin polypeptide or with maltose binding protein. The polypeptide immunogen may be a full-length molecule or a portion thereof. If the polypeptide portion is "hapten-like," such portion may be advantageously joined or linked to a macromolecular carrier (such as keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA) or tetanus toxoid) for immunization.

Although polyclonal antibodies are typically raised in animals such as horses, cows, dogs, chicken, rats, mice, rabbits, guinea pigs, goats, or sheep, an anti-Zcytor21 antibody of the present invention may also be derived from a subhuman primate antibody. General techniques for raising diagnostically and therapeutically

useful antibodies in baboons may be found, for example, in Goldenberg *et al.*, international patent publication No. WO 91/11465, and in Losman *et al.*, *Int. J. Cancer* 46:310 (1990).

Alternatively, monoclonal anti-Zcytor21 antibodies can be generated. Rodent monoclonal antibodies to specific antigens may be obtained by methods known to those skilled in the art (see, for example, Kohler *et al.*, *Nature* 256:495 (1975), Coligan *et al.* (eds.), *Current Protocols in Immunology*, Vol. 1, pages 2.5.1-2.6.7 (John Wiley & Sons 1991) ["Coligan"], Picksley *et al.*, "Production of monoclonal antibodies against proteins expressed in *E. coli*," in *DNA Cloning 2: Expression Systems*, 2nd Edition, Glover *et al.* (eds.), page 93 (Oxford University Press 1995)).

Briefly, monoclonal antibodies can be obtained by injecting mice with a composition comprising a *Zcytor21* gene product, verifying the presence of antibody production by removing a serum sample, removing the spleen to obtain B-lymphocytes, fusing the B-lymphocytes with myeloma cells to produce hybridomas, cloning the hybridomas, selecting positive clones which produce antibodies to the antigen, culturing the clones that produce antibodies to the antigen, and isolating the antibodies from the hybridoma cultures.

In addition, an anti-Zcytor21 antibody of the present invention may be derived from a human monoclonal antibody. Human monoclonal antibodies are obtained from transgenic mice that have been engineered to produce specific human antibodies in response to antigenic challenge. In this technique, elements of the human heavy and light chain locus are introduced into strains of mice derived from embryonic stem cell lines that contain targeted disruptions of the endogenous heavy chain and light chain loci. The transgenic mice can synthesize human antibodies specific for human antigens, and the mice can be used to produce human antibody-secreting hybridomas. Methods for obtaining human antibodies from transgenic mice are described, for example, by Green *et al.*, *Nature Genet.* 7:13 (1994), Lonberg *et al.*, *Nature* 368:856 (1994), and Taylor *et al.*, *Int. Immun.* 6:579 (1994).

Monoclonal antibodies can be isolated and purified from hybridoma cultures by a variety of well-established techniques. Such isolation techniques include affinity chromatography with Protein-A Sepharose, size-exclusion chromatography, and

ion-exchange chromatography (see, for example, Coligan at pages 2.7.1-2.7.12 and pages 2.9.1-2.9.3; Baines *et al.*, "Purification of Immunoglobulin G (IgG)," in *Methods in Molecular Biology*, Vol. 10, pages 79-104 (The Humana Press, Inc. 1992)).

For particular uses, it may be desirable to prepare fragments of anti-  
5 Zcytor21 antibodies. Such antibody fragments can be obtained, for example, by proteolytic hydrolysis of the antibody. Antibody fragments can be obtained by pepsin or papain digestion of whole antibodies by conventional methods. As an illustration, antibody fragments can be produced by enzymatic cleavage of antibodies with pepsin to provide a 5S fragment denoted F(ab')<sub>2</sub>. This fragment can be further cleaved using a  
10 thiol reducing agent to produce 3.5S Fab' monovalent fragments. Optionally, the cleavage reaction can be performed using a blocking group for the sulfhydryl groups that result from cleavage of disulfide linkages. As an alternative, an enzymatic cleavage using pepsin produces two monovalent Fab fragments and an Fc fragment directly. These methods are described, for example, by Goldenberg, U.S. patent No.  
15 4,331,647, Nisonoff *et al.*, *Arch Biochem. Biophys.* 89:230 (1960), Porter, *Biochem. J.* 73:119 (1959), Edelman *et al.*, in *Methods in Enzymology Vol. 1*, page 422 (Academic Press 1967), and by Coligan at pages 2.8.1-2.8.10 and 2.10.-2.10.4.

Other methods of cleaving antibodies, such as separation of heavy chains to form monovalent light-heavy chain fragments, further cleavage of fragments, or other  
20 enzymatic, chemical or genetic techniques may also be used, so long as the fragments bind to the antigen that is recognized by the intact antibody.

For example, Fv fragments comprise an association of V<sub>H</sub> and V<sub>L</sub> chains. This association can be noncovalent, as described by Inbar *et al.*, *Proc. Nat'l Acad. Sci. USA* 69:2659 (1972). Alternatively, the variable chains can be linked by an  
25 intermolecular disulfide bond or cross-linked by chemicals such as glutaraldehyde (see, for example, Sandhu, *Crit. Rev. Biotech.* 12:437 (1992)).

The Fv fragments may comprise V<sub>H</sub> and V<sub>L</sub> chains, which are connected by a peptide linker. These single-chain antigen binding proteins (scFv) are prepared by constructing a structural gene comprising DNA sequences encoding the V<sub>H</sub> and V<sub>L</sub>  
30 domains which are connected by an oligonucleotide. The structural gene is inserted into an expression vector, which is subsequently introduced into a host cell, such as *E.*

*coli*. The recombinant host cells synthesize a single polypeptide chain with a linker peptide bridging the two V domains. Methods for producing scFvs are described, for example, by Whitlow *et al.*, *Methods: A Companion to Methods in Enzymology* 2:97 (1991) (also see, Bird *et al.*, *Science* 242:423 (1988), Ladner *et al.*, U.S. Patent No. 4,946,778, Pack *et al.*, *Bio/Technology* 11:1271 (1993), and Sandhu, *supra*).

As an illustration, a scFV can be obtained by exposing lymphocytes to Zcytor21 polypeptide *in vitro*, and selecting antibody display libraries in phage or similar vectors (for instance, through use of immobilized or labeled Zcytor21 protein or peptide). Genes encoding polypeptides having potential Zcytor21 polypeptide binding domains can be obtained by screening random peptide libraries displayed on phage (phage display) or on bacteria, such as *E. coli*. Nucleotide sequences encoding the polypeptides can be obtained in a number of ways, such as through random mutagenesis and random polynucleotide synthesis. These random peptide display libraries can be used to screen for peptides, which interact with a known target which can be a protein or polypeptide, such as a ligand or receptor, a biological or synthetic macromolecule, or organic or inorganic substances. Techniques for creating and screening such random peptide display libraries are known in the art (Ladner *et al.*, U.S. Patent No. 5,223,409, Ladner *et al.*, U.S. Patent No. 4,946,778, Ladner *et al.*, U.S. Patent No. 5,403,484, Ladner *et al.*, U.S. Patent No. 5,571,698, and Kay *et al.*, *Phage Display of Peptides and Proteins* (Academic Press, Inc. 1996)) and random peptide display libraries and kits for screening such libraries are available commercially, for instance from CLONTECH Laboratories, Inc. (Palo Alto, CA), Invitrogen Inc. (San Diego, CA), New England Biolabs, Inc. (Beverly, MA), and Pharmacia LKB Biotechnology Inc. (Piscataway, NJ). Random peptide display libraries can be screened using the Zcytor21 sequences disclosed herein to identify proteins which bind to Zcytor21.

Another form of an antibody fragment is a peptide coding for a single complementarity-determining region (CDR). CDR peptides ("minimal recognition units") can be obtained by constructing genes encoding the CDR of an antibody of interest. Such genes are prepared, for example, by using the polymerase chain reaction to synthesize the variable region from RNA of antibody-producing cells (see, for example, Larrick *et al.*, *Methods: A Companion to Methods in Enzymology* 2:106

(1991), Courtenay-Luck, "Genetic Manipulation of Monoclonal Antibodies," in *Monoclonal Antibodies: Production, Engineering and Clinical Application*, Ritter *et al.* (eds.), page 166 (Cambridge University Press 1995), and Ward *et al.*, "Genetic Manipulation and Expression of Antibodies," in *Monoclonal Antibodies: Principles and Applications*, Birch *et al.*, (eds.), page 137 (Wiley-Liss, Inc. 1995)).

Alternatively, an anti-Zcytor21 antibody may be derived from a "humanized" monoclonal antibody. Humanized monoclonal antibodies are produced by transferring mouse complementary determining regions from heavy and light variable chains of the mouse immunoglobulin into a human variable domain. Typical residues of human antibodies are then substituted in the framework regions of the murine counterparts. The use of antibody components derived from humanized monoclonal antibodies obviates potential problems associated with the immunogenicity of murine constant regions. General techniques for cloning murine immunoglobulin variable domains are described, for example, by Orlandi *et al.*, *Proc. Nat'l Acad. Sci. USA* 86:3833 (1989). Techniques for producing humanized monoclonal antibodies are described, for example, by Jones *et al.*, *Nature* 321:522 (1986), Carter *et al.*, *Proc. Nat'l Acad. Sci. USA* 89:4285 (1992), Sandhu, *Crit. Rev. Biotech.* 12:437 (1992), Singer *et al.*, *J. Immun.* 150:2844 (1993), Sudhir (ed.), *Antibody Engineering Protocols* (Humana Press, Inc. 1995), Kelley, "Engineering Therapeutic Antibodies," in *Protein Engineering: Principles and Practice*, Cleland *et al.* (eds.), pages 399-434 (John Wiley & Sons, Inc. 1996), and by Queen *et al.*, U.S. Patent No. 5,693,762 (1997).

Polyclonal anti-idiotypic antibodies can be prepared by immunizing animals with anti-Zcytor21 antibodies or antibody fragments, using standard techniques. See, for example, Green *et al.*, "Production of Polyclonal Antisera," in *Methods In Molecular Biology: Immunochemical Protocols*, Manson (ed.), pages 1-12 (Humana Press 1992). Also, see Coligan at pages 2.4.1-2.4.7. Alternatively, monoclonal anti-idiotypic antibodies can be prepared using anti-Zcytor21 antibodies or antibody fragments as immunogens with the techniques, described above. As another alternative, humanized anti-idiotypic antibodies or subhuman primate anti-idiotypic antibodies can be prepared using the above-described techniques. Methods for producing anti-idiotypic antibodies are described, for example, by Irie, U.S. Patent No.

5,208,146, Greene, *et. al.*, U.S. Patent No. 5,637,677, and Varthakavi and Minocha, *J. Gen. Virol.* 77:1875 (1996).

9. *Use of Zcytor21 Nucleotide Sequences to Detect Gene Expression and Gene*  
5 *Structure*

Nucleic acid molecules can be used to detect the expression of a *Zcytor21* gene in a biological sample. Suitable probe molecules include double-stranded nucleic acid molecules comprising the nucleotide sequence of SEQ ID NOs:1, 4, 7, 10, or 14, or a portion thereof, as well as single-stranded nucleic acid molecules  
10 having the complement of the nucleotide sequence of SEQ ID NOs:1, 4, 7, 10, or 14, or a portion thereof. Probe molecules may be DNA, RNA; oligonucleotides, and the like. As used herein, the term "portion" refers to at least eight nucleotides to at least 20 or more nucleotides. Illustrative probes bind with regions of the *Zcytor21* gene that have a low sequence similarity to comparable regions in other cytokine receptor genes.

15 In a basic assay, a single-stranded probe molecule is incubated with RNA, isolated from a biological sample, under conditions of temperature and ionic strength that promote base pairing between the probe and target *Zcytor21* RNA species. After separating unbound probe from hybridized molecules, the amount of hybrids is detected.

20 Well-established hybridization methods of RNA detection include northern analysis and dot/slot blot hybridization (see, for example, Ausubel (1995) at pages 4-1 to 4-27, and Wu *et al.* (eds.), "Analysis of Gene Expression at the RNA Level," in *Methods in Gene Biotechnology*, pages 225-239 (CRC Press, Inc. 1997)). Nucleic acid probes can be detectably labeled with radioisotopes such as <sup>32</sup>P or <sup>35</sup>S.  
25 Alternatively, *Zcytor21* RNA can be detected with a nonradioactive hybridization method (see, for example, Isaac (ed.), *Protocols for Nucleic Acid Analysis by Nonradioactive Probes* (Humana Press, Inc. 1993)). Typically, nonradioactive detection is achieved by enzymatic conversion of chromogenic or chemiluminescent substrates. Illustrative nonradioactive moieties include biotin, fluorescein, and digoxigenin.

30 *Zcytor21* oligonucleotide probes are also useful for *in vivo* diagnosis. As an illustration, <sup>18</sup>F-labeled oligonucleotides can be administered to a subject and

visualized by positron emission tomography (Tavitian *et al.*, *Nature Medicine* 4:467 (1998)).

Numerous diagnostic procedures take advantage of the polymerase chain reaction (PCR) to increase sensitivity of detection methods. Standard techniques for performing PCR are well-known (see, generally, Mathew (ed.), *Protocols in Human Molecular Genetics* (Humana Press, Inc. 1991), White (ed.), *PCR Protocols: Current Methods and Applications* (Humana Press, Inc. 1993), Cotter (ed.), *Molecular Diagnosis of Cancer* (Humana Press, Inc. 1996), Hanausek and Walaszek (eds.), *Tumor Marker Protocols* (Humana Press, Inc. 1998), Lo (ed.), *Clinical Applications of PCR* (Humana Press, Inc. 1998), and Meltzer (ed.), *PCR in Bioanalysis* (Humana Press, Inc. 1998)).

PCR primers can be designed to amplify a portion of the *Zcytor21* gene that has a low sequence similarity to a comparable region in other proteins, such as other cytokine receptor proteins.

One variation of PCR for diagnostic assays is reverse transcriptase-PCR (RT-PCR). In the RT-PCR technique, RNA is isolated from a biological sample, reverse transcribed to cDNA, and the cDNA is incubated with *Zcytor21* primers (see, for example, Wu *et al.* (eds.), "Rapid Isolation of Specific cDNAs or Genes by PCR," in *Methods in Gene Biotechnology*, pages 15-28 (CRC Press, Inc. 1997)). PCR is then performed and the products are analyzed using standard techniques.

As an illustration, RNA is isolated from biological sample using, for example, the guanidinium-thiocyanate cell lysis procedure described above. Alternatively, a solid-phase technique can be used to isolate mRNA from a cell lysate. A reverse transcription reaction can be primed with the isolated RNA using random oligonucleotides, short homopolymers of dT, or *Zcytor21* anti-sense oligomers. Oligo-dT primers offer the advantage that various mRNA nucleotide sequences are amplified that can provide control target sequences. *Zcytor21* sequences are amplified by the polymerase chain reaction using two flanking oligonucleotide primers that are typically 20 bases in length.

PCR amplification products can be detected using a variety of approaches. For example, PCR products can be fractionated by gel electrophoresis, and



visualized by ethidium bromide staining. Alternatively, fractionated PCR products can be transferred to a membrane, hybridized with a detectably-labeled Zcytor21 probe, and examined by autoradiography. Additional alternative approaches include the use of digoxigenin-labeled deoxyribonucleic acid triphosphates to provide chemiluminescence  
5 detection, and the C-TRAK colorimetric assay.

Another approach for detection of *Zcytor21* expression is cycling probe technology, in which a single-stranded DNA target binds with an excess of DNA-RNA-DNA chimeric probe to form a complex, the RNA portion is cleaved with RNAase H, and the presence of cleaved chimeric probe is detected (see, for example, Beggs *et al.*,  
10 *J. Clin. Microbiol.* 34:2985 (1996), Bekkaoui *et al.*, *Biotechniques* 20:240 (1996)). Alternative methods for detection of *Zcytor21* sequences can utilize approaches such as nucleic acid sequence-based amplification, cooperative amplification of templates by cross-hybridization, and the ligase chain reaction (see, for example, Marshall *et al.*, U.S. Patent No. 5,686,272 (1997), Dyer *et al.*, *J. Virol. Methods* 60:161 (1996), Ehrlich  
15 *et al.*, *Eur. J. Biochem.* 243:358 (1997), and Chadwick *et al.*, *J. Virol. Methods* 70:59 (1998)). Other standard methods are known to those of skill in the art.

*Zcytor21* probes and primers can also be used to detect and to localize *Zcytor21* gene expression in tissue samples. Methods for such *in situ* hybridization are well-known to those of skill in the art (see, for example, Choo (ed.), *In Situ*  
20 *Hybridization Protocols* (Humana Press, Inc. 1994), Wu *et al.* (eds.), "Analysis of Cellular DNA or Abundance of mRNA by Radioactive *In Situ* Hybridization (RISH)," in *Methods in Gene Biotechnology*, pages 259-278 (CRC Press, Inc. 1997), and Wu *et al.* (eds.), "Localization of DNA or Abundance of mRNA by Fluorescence *In Situ* Hybridization (RISH)," in *Methods in Gene Biotechnology*, pages 279-289 (CRC Press,  
25 Inc. 1997)). Various additional diagnostic approaches are well-known to those of skill in the art (see, for example, Mathew (ed.), *Protocols in Human Molecular Genetics* (Humana Press, Inc. 1991), Coleman and Tsongalis, *Molecular Diagnostics* (Humana Press, Inc. 1996), and Elles, *Molecular Diagnosis of Genetic Diseases* (Humana Press, Inc., 1996)). Suitable test samples include blood, urine, saliva, tissue biopsy, and autopsy  
30 material.

Mutations of cytokine receptors are associated with particular diseases. For example, polymorphisms of cytokine receptors are associated with pulmonary alveolar proteinosis, familial periodic fever, and erythroleukemia. The *Zcytor21* gene is located in human chromosome 3p25.3. This region is associated with various disorders and diseases, including Fanconi anemia, xeroderma pigmentosum, a Marfan-like connective tissue disorder, and cardiomyopathy. Thus, *Zcytor21* nucleotide sequences can be used in linkage-based testing for various diseases, and to determine whether a subject's chromosomes contain a mutation in the *Zcytor21* gene. Detectable chromosomal aberrations at the *Zcytor21* gene locus include, but are not limited to, aneuploidy, gene copy number changes, insertions, deletions, restriction site changes and rearrangements. Of particular interest are genetic alterations that inactivate a *Zcytor21* gene.

The present invention also provides reagents which will find use in diagnostic applications. For example, the *zcytor21* gene, a probe comprising *zcytor21* DNA or RNA or a subsequence thereof can be used to determine if the *zcytor21* gene is present on a human chromosome, such as chromosome 3, or if a gene mutation has occurred. Based on annotation of a fragment of human genomic DNA containing a part of *zcytor21* genomic DNA, *zcytor21* is located at the p25.3 region of chromosome 3. Detectable chromosomal aberrations at the *zcytor21* gene locus include, but are not limited to, aneuploidy, gene copy number changes, loss of heterozygosity (LOH), translocations, insertions, deletions, restriction site changes and rearrangements. Such aberrations can be detected using polynucleotides of the present invention by employing molecular genetic techniques, such as restriction fragment length polymorphism (RFLP) analysis, short tandem repeat (STR) analysis employing PCR techniques, and other genetic linkage analysis techniques known in the art (Sambrook et al., *ibid.*; Ausubel et al., *ibid.*; Marian, *Chest* 108:255-65, 1995).

The precise knowledge of a gene's position can be useful for a number of purposes, including: 1) determining if a sequence is part of an existing contig and obtaining additional surrounding genetic sequences in various forms, such as YACs, BACs or cDNA clones; 2) providing a possible candidate gene for an inheritable disease which shows linkage to the same chromosomal region; and 3) cross-referencing

model organisms, such as mouse, which may aid in determining what function a particular gene might have.

The zcytor21 gene is located at the p25.3 region of chromosome 3. Several genes of known function map to this region that are linked to human disease. Thus, since the zcytor21 gene maps to chromosome p25.3, the zcytor21 polynucleotide probes of the present invention can be used to detect and diagnose the presence of chromosome 3 monosomy and other chromosome p25.3 loss, and particularly chromosome 3 monosomy and loss and chromosomal aberrations at p25.3 including deletions, rearrangements, and chromosomal breakpoints, and translocations can be associated with tumors. Thus, since the zcytor21 gene maps to this critical region, the zcytor21 polynucleotide probes of the present invention can be used to detect chromosome deletions, translocations and rearrangements associated with those diseases. See the Online Mendellian Inheritance of Man (OMIM™, National Center for Biotechnology Information, National Library of Medicine, Bethesda, MD) gene map, and references therein, for this region of human chromosome 3, and p25.3 on a publicly available world wide web server. All of these serve as possible candidate genes for an inheritable disease that show linkage to the same chromosomal region as the zcytor21 gene. Thus, zcytor21 polynucleotide probes can be used to detect abnormalities or genotypes associated with these defects.

A diagnostic could assist physicians in determining the type of disease and appropriate associated therapy, or assistance in genetic counseling. As such, the inventive anti-zcytor21 antibodies, polynucleotides, and polypeptides can be used for the detection of zcytor21 polypeptide, mRNA or anti-zcytor21 antibodies, thus serving as markers and be directly used for detecting or genetic diseases or cancers, as described herein, using methods known in the art and described herein. Further, zcytor21 polynucleotide probes can be used to detect abnormalities or genotypes associated with chromosome p25.3 deletions, chromosome 3 monosomy and translocations associated with human diseases, such as described above, or other translocations and LOH involved with malignant progression of tumors or other p25.3 mutations, which are expected to be involved in chromosome rearrangements in malignancy; or in other cancers. Similarly, zcytor21 polynucleotide probes can be used

to detect abnormalities or genotypes associated with chromosome p25.3 trisomy and chromosome loss associated with human diseases or spontaneous abortion. All of these serve as possible candidate genes for an inheritable disease which show linkage to the same chromosomal region as the zcytor21 gene. Thus, zcytor21 polynucleotide probes  
5 can be used to detect abnormalities or genotypes associated with these defects.

One of skill in the art would recognize that of zcytor21 polynucleotide probes are particularly useful for diagnosis of gross chromosome 3 abnormalities associated with loss of heterogeneity (LOH), chromosome gain (e.g. trisomy), translocation, chromosome loss (monosomy), DNA amplification, and the like.  
10 Translocations within chromosomal locus p25.3 wherein the zcytor21 gene is located are known to be associated with human disease. For example, p25.3 deletions, monosomy and translocations are associated with specific human diseases as discussed above. Thus, since the zcytor21 gene maps to this critical region, zcytor21 polynucleotide probes of the present invention can be used to detect abnormalities or  
15 genotypes associated with p25.3 translocation, deletion and trisomy, and the like, described above.

As discussed above, defects in the zcytor21 gene itself may result in a heritable human disease state. Molecules of the present invention, such as the polypeptides, antagonists, agonists, polynucleotides and antibodies of the present  
20 invention would aid in the detection, diagnosis prevention, and treatment associated with a zcytor21 genetic defect. In addition, zcytor21 polynucleotide probes can be used to detect allelic differences between diseased or non-diseased individuals at the zcytor21 chromosomal locus. As such, the zcytor21 sequences can be used as diagnostics in forensic DNA profiling.

25 The protein truncation test is also useful for detecting the inactivation of a gene in which translation-terminating mutations produce only portions of the encoded protein (see, for example, Stoppa-Lyonnet *et al.*, *Blood* 91:3920 (1998)). According to this approach, RNA is isolated from a biological sample, and used to synthesize cDNA. PCR is then used to amplify the *Zcytor21* target sequence and to introduce an RNA  
30 polymerase promoter, a translation initiation sequence, and an in-frame ATG triplet. PCR products are transcribed using an RNA polymerase, and the transcripts are

translated *in vitro* with a T7-coupled reticulocyte lysate system. The translation products are then fractionated by SDS-PAGE to determine the lengths of the translation products. The protein truncation test is described, for example, by Dracopoli *et al.* (eds.), *Current Protocols in Human Genetics*, pages 9.11.1 - 9.11.18 (John Wiley & Sons 1998).

The present invention also contemplates kits for performing a diagnostic assay for *Zcytor21* gene expression or to detect mutations in the *Zcytor21* gene. Such kits comprise nucleic acid probes, such as double-stranded nucleic acid molecules comprising the nucleotide sequence of nucleotides 135 to 1427 of SEQ ID NO:10, the nucleotide sequence of SEQ ID NO:10, or a portion thereof, as well as single-stranded nucleic acid molecules having the complement of the nucleotide sequence of SEQ ID NO:10, or a portion thereof. Nucleic acid probes can also be based upon the nucleotide sequences of SEQ ID NOs: 1, 4, 7, or 14. Probe molecules may be DNA, RNA, oligonucleotides, and the like. Kits may comprise nucleic acid primers for performing PCR.

Such kits can contain all the elements to perform a nucleic acid diagnostic assay described above. A kit will comprise at least one container comprising a *Zcytor21* probe or primer. The kit may also comprise a second container comprising one or more reagents capable of indicating the presence of *Zcytor21* sequences. Examples of such indicator reagents include detectable labels such as radioactive labels, fluorochromes, chemiluminescent agents, and the like. A kit may also comprise a means for conveying to the user that the *Zcytor21* probes and primers are used to detect *Zcytor21* gene expression. For example, written instructions may state that the enclosed nucleic acid molecules can be used to detect either a nucleic acid molecule that encodes *Zcytor21*, or a nucleic acid molecule having a nucleotide sequence that is complementary to a *Zcytor21*-encoding nucleotide sequence. The written material can be applied directly to a container, or the written material can be provided in the form of a packaging insert.

#### 10. Use of Anti-Zcytor21 Antibodies to Detect Zcytor21

The present invention contemplates the use of anti-Zcytor21 antibodies to screen biological samples *in vitro* for the presence of Zcytor21. In one type of *in vitro* assay, anti-Zcytor21 antibodies are used in liquid phase. For example, the presence of Zcytor21 in a biological sample can be tested by mixing the biological sample with a trace amount of labeled Zcytor21 and an anti-Zcytor21 antibody under conditions that promote binding between Zcytor21 and its antibody. Complexes of Zcytor21 and anti-Zcytor21 in the sample can be separated from the reaction mixture by contacting the complex with an immobilized protein which binds with the antibody, such as an Fc antibody or *Staphylococcus* protein A. The concentration of Zcytor21 in the biological sample will be inversely proportional to the amount of labeled Zcytor21 bound to the antibody and directly related to the amount of free-labeled Zcytor21. Illustrative biological samples include blood, urine, saliva, tissue biopsy, and autopsy material.

Alternatively, *in vitro* assays can be performed in which anti-Zcytor21 antibody is bound to a solid-phase carrier. For example, antibody can be attached to a polymer, such as aminodextran, in order to link the antibody to an insoluble support such as a polymer-coated bead, a plate or a tube. Other suitable *in vitro* assays will be readily apparent to those of skill in the art.

In another approach, anti-Zcytor21 antibodies can be used to detect Zcytor21 in tissue sections prepared from a biopsy specimen. Such immunochemical detection can be used to determine the relative abundance of Zcytor21 and to determine the distribution of Zcytor21 in the examined tissue. General immunochemistry techniques are well established (see, for example, Ponder, "Cell Marking Techniques and Their Application," in *Mammalian Development: A Practical Approach*, Monk (ed.), pages 115-38 (IRL Press 1987), Coligan at pages 5.8.1-5.8.8, Ausubel (1995) at pages 14.6.1 to 14.6.13 (Wiley Interscience 1990), and Manson (ed.), *Methods In Molecular Biology, Vol. 10: Immunochemical Protocols* (The Humana Press, Inc. 1992)).

Immunochemical detection can be performed by contacting a biological sample with an anti-Zcytor21 antibody, and then contacting the biological sample with a detectably labeled molecule, which binds to the antibody. For example, the detectably labeled molecule can comprise an antibody moiety that binds to anti-Zcytor21 antibody.

Alternatively, the anti-Zcytor21 antibody can be conjugated with avidin/streptavidin (or biotin) and the detectably labeled molecule can comprise biotin (or avidin/streptavidin). Numerous variations of this basic technique are well-known to those of skill in the art.

Alternatively, an anti-Zcytor21 antibody can be conjugated with a detectable label to form an anti-Zcytor21 immunoconjugate. Suitable detectable labels include, for example, a radioisotope, a fluorescent label, a chemiluminescent label, an enzyme label, a bioluminescent label or colloidal gold. Methods of making and detecting such detectably-labeled immunoconjugates are well-known to those of ordinary skill in the art, and are described in more detail below.

The detectable label can be a radioisotope that is detected by autoradiography. Isotopes that are particularly useful for the purpose of the present invention are  $^3\text{H}$ ,  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{35}\text{S}$  and  $^{14}\text{C}$ .

Anti-Zcytor21 immunoconjugates can also be labeled with a fluorescent compound. The presence of a fluorescently-labeled antibody is determined by exposing the immunoconjugate to light of the proper wavelength and detecting the resultant fluorescence. Fluorescent labeling compounds include fluorescein isothiocyanate, rhodamine, phycoerytherin, phycocyanin, allophycocyanin, o-phthaldehyde and fluorescamine.

Alternatively, anti-Zcytor21 immunoconjugates can be detectably labeled by coupling an antibody component to a chemiluminescent compound. The presence of the chemiluminescent-tagged immunoconjugate is determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of chemiluminescent labeling compounds include luminol, isoluminol, an aromatic acridinium ester, an imidazole, an acridinium salt and an oxalate ester.

Similarly, a bioluminescent compound can be used to label anti-Zcytor21 immunoconjugates of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in which a catalytic protein increases the efficiency of the chemiluminescent reaction. The presence of a bioluminescent protein is determined by detecting the presence of luminescence. Bioluminescent compounds that are useful for labeling include luciferin, luciferase and aequorin.

Alternatively, anti-Zcytor21 immunoconjugates can be detectably labeled by linking an anti-Zcytor21 antibody component to an enzyme. When the anti-Zcytor21-

enzyme conjugate is incubated in the presence of the appropriate substrate, the enzyme moiety reacts with the substrate to produce a chemical moiety, which can be detected, for example, by spectrophotometric, fluorometric or visual means. Examples of enzymes that can be used to detectably label polyspecific immunoconjugates include  $\beta$ -galactosidase, glucose oxidase, peroxidase and alkaline phosphatase.

Those of skill in the art will know of other suitable labels, which can be employed in accordance with the present invention. The binding of marker moieties to anti-Zcytor21 antibodies can be accomplished using standard techniques known to the art. Typical methodology in this regard is described by Kennedy *et al.*, *Clin. Chim. Acta* 70:1 (1976), Schurs *et al.*, *Clin. Chim. Acta* 81:1 (1977), Shih *et al.*, *Int'l J. Cancer* 46:1101 (1990), Stein *et al.*, *Cancer Res.* 50:1330 (1990), and Coligan, *supra*.

Moreover, the convenience and versatility of immunochemical detection can be enhanced by using anti-Zcytor21 antibodies that have been conjugated with avidin, streptavidin, and biotin (see, for example, Wilchek *et al.* (eds.), "Avidin-Biotin Technology," *Methods In Enzymology*, Vol. 184 (Academic Press 1990), and Bayer *et al.*, "Immunochemical Applications of Avidin-Biotin Technology," in *Methods In Molecular Biology*, Vol. 10, Manson (ed.), pages 149-162 (The Humana Press, Inc. 1992).

Methods for performing immunoassays are well-established. See, for example, Cook and Self, "Monoclonal Antibodies in Diagnostic Immunoassays," in *Monoclonal Antibodies: Production, Engineering, and Clinical Application*, Ritter and Ladyman (eds.), pages 180-208, (Cambridge University Press, 1995), Perry, "The Role of Monoclonal Antibodies in the Advancement of Immunoassay Technology," in *Monoclonal Antibodies: Principles and Applications*, Birch and Lennox (eds.), pages 107-120 (Wiley-Liss, Inc. 1995), and Diamandis, *Immunoassay* (Academic Press, Inc. 1996).

The present invention also contemplates kits for performing an immunological diagnostic assay for Zcytor21 gene expression. Such kits comprise at least one container comprising an anti-Zcytor21 antibody, or antibody fragment. A kit may also comprise a second container comprising one or more reagents capable of indicating the presence of Zcytor21 antibody or antibody fragments. Examples of such indicator reagents include detectable labels such as a radioactive label, a fluorescent



label, a chemiluminescent label, an enzyme label, a bioluminescent label, colloidal gold, and the like. A kit may also comprise a means for conveying to the user that Zcytor21 antibodies or antibody fragments are used to detect Zcytor21 protein. For example, written instructions may state that the enclosed antibody or antibody fragment can be used to detect Zcytor21. The written material can be applied directly to a container, or the written material can be provided in the form of a packaging insert.

#### 11. *Therapeutic Uses of Polypeptides Having Zcytor21 Activity*

Amino acid sequences having Zcytor21 activity can be used to modulate the immune system by binding Zcytor21 ligand, and thus, preventing the binding of Zcytor21 ligand with endogenous Zcytor21 receptor. As an illustration, polypeptides having Zcytor21 activity can be used to inhibit cell proliferation associated with, for example, psoriasis or the growth of a tumor (*e.g.*, a melanoma). Zcytor21 antagonists, such as anti-Zcytor21 antibodies, can also be used to modulate the immune system by inhibiting the binding of Zcytor21 ligand with the endogenous Zcytor21 receptor.

Accordingly, the present invention includes the use of proteins, polypeptides, and peptides having Zcytor21 activity (such as Zcytor21 polypeptides, Zcytor21 analogs (*e.g.*, anti-Zcytor21 anti-idiotypic antibodies), and Zcytor21 fusion proteins) to a subject which lacks an adequate amount of Zcytor21 polypeptide, or which produces an excess of Zcytor21 ligand. Zcytor21 antagonists (*e.g.*, anti-Zcytor21 antibodies) can be also used to treat a subject, which produces an excess of either Zcytor21 ligand or Zcytor21. These molecules can be administered to any subject in need of treatment, and the present invention contemplates both veterinary and human therapeutic uses. Illustrative subjects include mammalian subjects, such as farm animals, domestic animals, and human patients.

Generally, the dosage of administered Zcytor21 (or Zcytor21 analog or fusion protein) will vary depending upon such factors as the subject's age, weight, height, sex, general medical condition and previous medical history. Typically, it is desirable to provide the recipient with a dosage of Zcytor21 polypeptide, which is in the range of from about 1 pg/kg to 10 mg/kg (amount of agent/body weight of subject), although a lower or higher dosage also may be administered as circumstances dictate.

Administration of a Zcytor21 polypeptide to a subject can be intravenous, intraarterial, intraperitoneal, intramuscular, subcutaneous, intrapleural, intrathecal, by perfusion through a regional catheter, or by direct intralesional injection. When administering therapeutic proteins by injection, the administration may be by continuous infusion or by single or multiple boluses.

Additional routes of administration include oral, mucosal-membrane, pulmonary, and transcutaneous. Oral delivery is suitable for polyester microspheres, zein microspheres, proteinoid microspheres, polycyanoacrylate microspheres, and lipid-based systems (see, for example, DiBase and Morrel, "Oral Delivery of Microencapsulated Proteins," in *Protein Delivery: Physical Systems*, Sanders and Hendren (eds.), pages 255-288 (Plenum Press 1997)). The feasibility of an intranasal delivery is exemplified by such a mode of insulin administration (see, for example, Hinchcliffe and Illum, *Adv. Drug Deliv. Rev.* 35:199 (1999)). Dry or liquid particles comprising Zcytor21 can be prepared and inhaled with the aid of dry-powder dispersers, liquid aerosol generators, or nebulizers (e.g., Pettit and Gombotz, *TIBTECH* 16:343 (1998); Patton *et al.*, *Adv. Drug Deliv. Rev.* 35:235 (1999)). This approach is illustrated by the AERX diabetes management system, which is a hand-held electronic inhaler that delivers aerosolized insulin into the lungs. Studies have shown that proteins as large as 48,000 kDa have been delivered across skin at therapeutic concentrations with the aid of low-frequency ultrasound, which illustrates the feasibility of transcutaneous administration (Mitragotri *et al.*, *Science* 269:850 (1995)). Transdermal delivery using electroporation provides another means to administer a molecule having Zcytor21 binding activity (Potts *et al.*, *Pharm. Biotechnol.* 10:213 (1997)).

A pharmaceutical composition comprising a protein, polypeptide, or peptide having Zcytor21 binding activity can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby the therapeutic proteins are combined in a mixture with a pharmaceutically acceptable carrier. A composition is said to be a "pharmaceutically acceptable carrier" if its administration can be tolerated by a recipient patient. Sterile phosphate-buffered saline is one example of a pharmaceutically acceptable carrier. Other suitable carriers are well-known to

those in the art. See, for example, Gennaro (ed.), *Remington's Pharmaceutical Sciences*, 19th Edition (Mack Publishing Company 1995).

For purposes of therapy, molecules having Zcytor21 binding activity and a pharmaceutically acceptable carrier are administered to a patient in a therapeutically effective amount. A combination of a protein, polypeptide, or peptide having Zcytor21 binding activity and a pharmaceutically acceptable carrier is said to be administered in a "therapeutically effective amount" if the amount administered is physiologically significant. An agent is physiologically significant if its presence results in a detectable change in the physiology of a recipient patient. For example, an agent used to treat inflammation is physiologically significant if its presence alleviates the inflammatory response. As another example, an agent used to inhibit the growth of tumor cells is physiologically significant if the administration of the agent results in a decrease in the number of tumor cells, decreased metastasis, a decrease in the size of a solid tumor, or increased necrosis of a tumor.

A pharmaceutical composition comprising Zcytor21 (or Zcytor21 analog or fusion protein) can be furnished in liquid form, in an aerosol, or in solid form. Liquid forms, are illustrated by injectable solutions and oral suspensions. Exemplary solid forms include capsules, tablets, and controlled-release forms. The latter form is illustrated by miniosmotic pumps and implants (Bremer *et al.*, *Pharm. Biotechnol.* 10:239 (1997); Ranade, "Implants in Drug Delivery," in *Drug Delivery Systems*, Ranade and Hollinger (eds.), pages 95-123 (CRC Press 1995); Bremer *et al.*, "Protein Delivery with Infusion Pumps," in *Protein Delivery: Physical Systems*, Sanders and Hendren (eds.), pages 239-254 (Plenum Press 1997); Yewey *et al.*, "Delivery of Proteins from a Controlled Release Injectable Implant," in *Protein Delivery: Physical Systems*, Sanders and Hendren (eds.), pages 93-117 (Plenum Press 1997)).

Liposomes provide one means to deliver therapeutic polypeptides to a subject intravenously, intraperitoneally, intrathecally, intramuscularly, subcutaneously, or via oral administration, inhalation, or intranasal administration. Liposomes are microscopic vesicles that consist of one or more lipid bilayers surrounding aqueous compartments (see, generally, Bakker-Woudenberg *et al.*, *Eur. J. Clin. Microbiol. Infect. Dis.* 12 (Suppl. 1):S61 (1993), Kim, *Drugs* 46:618 (1993), and Ranade, "Site-

Specific Drug Delivery Using Liposomes as Carriers," in *Drug Delivery Systems*, Ranade and Hollinger (eds.), pages 3-24 (CRC Press 1995)). Liposomes are similar in composition to cellular membranes and as a result, liposomes can be administered safely and are biodegradable. Depending on the method of preparation, liposomes may be unilamellar or multilamellar, and liposomes can vary in size with diameters ranging from 0.02  $\mu\text{m}$  to greater than 10  $\mu\text{m}$ . A variety of agents can be encapsulated in liposomes: hydrophobic agents partition in the bilayers and hydrophilic agents partition within the inner aqueous space(s) (see, for example, Machy *et al.*, *Liposomes In Cell Biology And Pharmacology* (John Libbey 1987), and Ostro *et al.*, *American J. Hosp. Pharm.* 46:1576 (1989)). Moreover, it is possible to control the therapeutic availability of the encapsulated agent by varying liposome size, the number of bilayers, lipid composition, as well as the charge and surface characteristics of the liposomes.

Liposomes can adsorb to virtually any type of cell and then slowly release the encapsulated agent. Alternatively, an absorbed liposome may be endocytosed by cells that are phagocytic. Endocytosis is followed by intralysosomal degradation of liposomal lipids and release of the encapsulated agents (Scherphof *et al.*, *Ann. N.Y. Acad. Sci.* 446:368 (1985)). After intravenous administration, small liposomes (0.1 to 1.0  $\mu\text{m}$ ) are typically taken up by cells of the reticuloendothelial system, located principally in the liver and spleen, whereas liposomes larger than 3.0  $\mu\text{m}$  are deposited in the lung. This preferential uptake of smaller liposomes by the cells of the reticuloendothelial system has been used to deliver chemotherapeutic agents to macrophages and to tumors of the liver.

The reticuloendothelial system can be circumvented by several methods including saturation with large doses of liposome particles, or selective macrophage inactivation by pharmacological means (Claassen *et al.*, *Biochim. Biophys. Acta* 802:428 (1984)). In addition, incorporation of glycolipid- or polyethylene glycol-derivatized phospholipids into liposome membranes has been shown to result in a significantly reduced uptake by the reticuloendothelial system (Allen *et al.*, *Biochim. Biophys. Acta* 1068:133 (1991); Allen *et al.*, *Biochim. Biophys. Acta* 1150:9 (1993)).

Liposomes can also be prepared to target particular cells or organs by varying phospholipid composition or by inserting receptors or ligands into the

liposomes. For example, liposomes, prepared with a high content of a nonionic surfactant, have been used to target the liver (Hayakawa *et al.*, Japanese Patent 04-244,018; Kato *et al.*, *Biol. Pharm. Bull.* 16:960 (1993)). These formulations were prepared by mixing soybean phosphatidylcholine,  $\alpha$ -tocopherol, and ethoxylated hydrogenated castor oil (HCO-60) in methanol, concentrating the mixture under vacuum, and then reconstituting the mixture with water. A liposomal formulation of dipalmitoylphosphatidylcholine (DPPC) with a soybean-derived sterylglucoside mixture (SG) and cholesterol (Ch) has also been shown to target the liver (Shimizu *et al.*, *Biol. Pharm. Bull.* 20:881 (1997)).

Alternatively, various targeting ligands can be bound to the surface of the liposome, such as antibodies, antibody fragments, carbohydrates, vitamins, and transport proteins. For example, liposomes can be modified with branched type galactosyllipid derivatives to target asialoglycoprotein (galactose) receptors, which are exclusively expressed on the surface of liver cells (Kato and Sugiyama, *Crit. Rev. Ther. Drug Carrier Syst.* 14:287 (1997); Murahashi *et al.*, *Biol. Pharm. Bull.* 20:259 (1997)). Similarly, Wu *et al.*, *Hepatology* 27:772 (1998), have shown that labeling liposomes with asialofetuin led to a shortened liposome plasma half-life and greatly enhanced uptake of asialofetuin-labeled liposome by hepatocytes. On the other hand, hepatic accumulation of liposomes comprising branched type galactosyllipid derivatives can be inhibited by preinjection of asialofetuin (Murahashi *et al.*, *Biol. Pharm. Bull.* 20:259 (1997)). Polyacetylated human serum albumin liposomes provide another approach for targeting liposomes to liver cells (Kamps *et al.*, *Proc. Nat'l Acad. Sci. USA* 94:11681 (1997)). Moreover, Geho, *et al.* U.S. Patent No. 4,603,044, describe a hepatocyte-directed liposome vesicle delivery system, which has specificity for hepatobiliary receptors associated with the specialized metabolic cells of the liver.

In a more general approach to tissue targeting, target cells are prelabeled with biotinylated antibodies specific for a ligand expressed by the target cell (Harasym *et al.*, *Adv. Drug Deliv. Rev.* 32:99 (1998)). After plasma elimination of free antibody, streptavidin-conjugated liposomes are administered. In another approach, targeting antibodies are directly attached to liposomes (Harasym *et al.*, *Adv. Drug Deliv. Rev.* 32:99 (1998)).

Polypeptides having Zcytor21 binding activity can be encapsulated within liposomes using standard techniques of protein microencapsulation (see, for example, Anderson *et al.*, *Infect. Immun.* 31:1099 (1981), Anderson *et al.*, *Cancer Res.* 50:1853 (1990), and Cohen *et al.*, *Biochim. Biophys. Acta* 1063:95 (1991), Alving *et al.* 5 "Preparation and Use of Liposomes in Immunological Studies," in *Liposome Technology*, 2nd Edition, Vol. III, Gregoriadis (ed.), page 317 (CRC Press 1993), Wassef *et al.*, *Meth. Enzymol.* 149:124 (1987)). As noted above, therapeutically useful liposomes may contain a variety of components. For example, liposomes may comprise lipid derivatives of poly(ethylene glycol) (Allen *et al.*, *Biochim. Biophys. Acta* 1150:9 10 (1993)).

Degradable polymer microspheres have been designed to maintain high systemic levels of therapeutic proteins. Microspheres are prepared from degradable polymers such as poly(lactide-co-glycolide) (PLG), polyanhydrides, poly (ortho esters), nonbiodegradable ethylvinyl acetate polymers, in which proteins are entrapped in the polymer (Gombotz and Pettit, *Bioconjugate Chem.* 6:332 (1995); Ranade, "Role of 15 Polymers in Drug Delivery," in *Drug Delivery Systems*, Ranade and Hollinger (eds.), pages 51-93 (CRC Press 1995); Roskos and Maskiewicz, "Degradable Controlled Release Systems Useful for Protein Delivery," in *Protein Delivery: Physical Systems*, Sanders and Hendren (eds.), pages 45-92 (Plenum Press 1997); Bartus *et al.*, *Science* 20 281:1161 (1998); Putney and Burke, *Nature Biotechnology* 16:153 (1998); Putney, *Curr. Opin. Chem. Biol.* 2:548 (1998)). Polyethylene glycol (PEG)-coated nanospheres can also provide carriers for intravenous administration of therapeutic proteins (see, for example, Gref *et al.*, *Pharm. Biotechnol.* 10:167 (1997)).

The present invention also contemplates chemically modified 25 polypeptides having binding Zcytor21 activity and Zcytor21 antagonists, in which a polypeptide is linked with a polymer, as discussed above.

Other dosage forms can be devised by those skilled in the art, as shown, for example, by Ansel and Popovich, *Pharmaceutical Dosage Forms and Drug Delivery Systems*, 5<sup>th</sup> Edition (Lea & Febiger 1990), Gennaro (ed.), *Remington's* 30 *Pharmaceutical Sciences*, 19<sup>th</sup> Edition (Mack Publishing Company 1995), and by Ranade and Hollinger, *Drug Delivery Systems* (CRC Press 1996).

As an illustration, pharmaceutical compositions may be supplied as a kit comprising a container that comprises a polypeptide with a Zcytor21 extracellular domain or a Zcytor21 antagonist (e.g., an antibody or antibody fragment that binds a Zcytor21 polypeptide). Therapeutic polypeptides can be provided in the form of an injectable solution for single or multiple doses, or as a sterile powder that will be reconstituted before injection. Alternatively, such a kit can include a dry-powder disperser, liquid aerosol generator, or nebulizer for administration of a therapeutic polypeptide. Such a kit may further comprise written information on indications and usage of the pharmaceutical composition. Moreover, such information may include a statement that the Zcytor21 composition is contraindicated in patients with known hypersensitivity to Zcytor21.

## 12. Therapeutic Uses of Zcytor21 Nucleotide Sequences

The present invention includes the use of Zcytor21 nucleotide sequences to provide Zcytor21 to a subject in need of such treatment. In addition, a therapeutic expression vector can be provided that inhibits Zcytor21 gene expression, such as an anti-sense molecule, a ribozyme, or an external guide sequence molecule.

There are numerous approaches to introduce a Zcytor21 gene to a subject, including the use of recombinant host cells that express Zcytor21, delivery of naked nucleic acid encoding Zcytor21, use of a cationic lipid carrier with a nucleic acid molecule that encodes Zcytor21, and the use of viruses that express Zcytor21, such as recombinant retroviruses, recombinant adeno-associated viruses, recombinant adenoviruses, and recombinant Herpes simplex viruses (see, for example, Mulligan, *Science* 260:926 (1993), Rosenberg *et al.*, *Science* 242:1575 (1988), LaSalle *et al.*, *Science* 259:988 (1993), Wolff *et al.*, *Science* 247:1465 (1990), Breakfield and Deluca, *The New Biologist* 3:203 (1991)). In an *ex vivo* approach, for example, cells are isolated from a subject, transfected with a vector that expresses a Zcytor21 gene, and then transplanted into the subject.

In order to effect expression of a Zcytor21 gene, an expression vector is constructed in which a nucleotide sequence encoding a Zcytor21 gene is operably linked

to a core promoter, and optionally a regulatory element, to control gene transcription. The general requirements of an expression vector are described above.

Alternatively, a *Zcytor21* gene can be delivered using recombinant viral vectors, including for example, adenoviral vectors (e.g., Kass-Eisler *et al.*, *Proc. Nat'l Acad. Sci. USA* 90:11498 (1993), Kolls *et al.*, *Proc. Nat'l Acad. Sci. USA* 91:215 (1994), Li *et al.*, *Hum. Gene Ther.* 4:403 (1993), Vincent *et al.*, *Nat. Genet.* 5:130 (1993), and Zabner *et al.*, *Cell* 75:207 (1993)), adenovirus-associated viral vectors (Flotte *et al.*, *Proc. Nat'l Acad. Sci. USA* 90:10613 (1993)), alphaviruses such as Semliki Forest Virus and Sindbis Virus (Hertz and Huang, *J. Vir.* 66:857 (1992), Raju and Huang, *J. Vir.* 65:2501 (1991), and Xiong *et al.*, *Science* 243:1188 (1989)), herpes viral vectors (e.g., U.S. Patent Nos. 4,769,331, 4,859,587, 5,288,641 and 5,328,688), parvovirus vectors (Koering *et al.*, *Hum. Gene Therap.* 5:457 (1994)), pox virus vectors (Ozaki *et al.*, *Biochem. Biophys. Res. Comm.* 193:653 (1993), Panicali and Paoletti, *Proc. Nat'l Acad. Sci. USA* 79:4927 (1982)), pox viruses, such as canary pox virus or vaccinia virus (Fisher-Hoch *et al.*, *Proc. Nat'l Acad. Sci. USA* 86:317 (1989), and Flexner *et al.*, *Ann. N.Y. Acad. Sci.* 569:86 (1989)), and retroviruses (e.g., Baba *et al.*, *J. Neurosurg* 79:729 (1993), Ram *et al.*, *Cancer Res.* 53:83 (1993), Takamiya *et al.*, *J. Neurosci. Res* 33:493 (1992), Vile and Hart, *Cancer Res.* 53:962 (1993), Vile and Hart, *Cancer Res.* 53:3860 (1993), and Anderson *et al.*, U.S. Patent No. 5,399,346). Within various embodiments, either the viral vector itself, or a viral particle which contains the viral vector may be utilized in the methods and compositions described below.

As an illustration of one system, adenovirus, a double-stranded DNA virus, is a well-characterized gene transfer vector for delivery of a heterologous nucleic acid molecule (for a review, see Becker *et al.*, *Meth. Cell Biol.* 43:161 (1994); Douglas and Curiel, *Science & Medicine* 4:44 (1997)). The adenovirus system offers several advantages including: (i) the ability to accommodate relatively large DNA inserts, (ii) the ability to be grown to high-titer, (iii) the ability to infect a broad range of mammalian cell types, and (iv) the ability to be used with many different promoters including ubiquitous, tissue specific, and regulatable promoters. In addition, adenoviruses can be administered by intravenous injection, because the viruses are stable in the bloodstream.



Using adenovirus vectors where portions of the adenovirus genome are deleted, inserts are incorporated into the viral DNA by direct ligation or by homologous recombination with a co-transfected plasmid. In an exemplary system, the essential E1 gene is deleted from the viral vector, and the virus will not replicate unless the E1 gene is provided by the host cell. When intravenously administered to intact animals, adenovirus primarily targets the liver. Although an adenoviral delivery system with an E1 gene deletion cannot replicate in the host cells, the host's tissue will express and process an encoded heterologous protein. Host cells will also secrete the heterologous protein if the corresponding gene includes a secretory signal sequence. Secreted proteins will enter the circulation from tissue that expresses the heterologous gene (e.g., the highly vascularized liver).

Moreover, adenoviral vectors containing various deletions of viral genes can be used to reduce or eliminate immune responses to the vector. Such adenoviruses are E1-deleted, and in addition, contain deletions of E2A or E4 (Lusky *et al.*, *J. Virol.* 72:2022 (1998); Raper *et al.*, *Human Gene Therapy* 9:671 (1998)). The deletion of E2b has also been reported to reduce immune responses (Amalfitano *et al.*, *J. Virol.* 72:926 (1998)). By deleting the entire adenovirus genome, very large inserts of heterologous DNA can be accommodated. The generation of so called "gutless" adenoviruses, where all viral genes are deleted, is particularly advantageous for insertion of large inserts of heterologous DNA (for a review, see Yeh. and Perricaudet, *FASEB J.* 11:615 (1997)).

High titer stocks of recombinant viruses capable of expressing a therapeutic gene can be obtained from infected mammalian cells using standard methods. For example, recombinant herpes simplex virus can be prepared in Vero cells, as described by Brandt *et al.*, *J. Gen. Virol.* 72:2043 (1991), Herold *et al.*, *J. Gen. Virol.* 75:1211 (1994), Visalli and Brandt, *Virology* 185:419 (1991), Grau *et al.*, *Invest. Ophthalmol. Vis. Sci.* 30:2474 (1989), Brandt *et al.*, *J. Virol. Meth.* 36:209 (1992), and by Brown and MacLean (eds.), *HSV Virus Protocols* (Humana Press 1997).

Alternatively, an expression vector comprising a *Zcytor21* gene can be introduced into a subject's cells by lipofection *in vivo* using liposomes. Synthetic cationic lipids can be used to prepare liposomes for *in vivo* transfection of a gene encoding a marker (Felgner *et al.*, *Proc. Nat'l Acad. Sci. USA* 84:7413 (1987); Mackey

*et al.*, *Proc. Nat'l Acad. Sci. USA* 85:8027 (1988)). The use of lipofection to introduce exogenous genes into specific organs *in vivo* has certain practical advantages. Liposomes can be used to direct transfection to particular cell types, which is particularly advantageous in a tissue with cellular heterogeneity, such as the pancreas, liver, kidney, and brain. Lipids may be chemically coupled to other molecules for the purpose of targeting. Targeted peptides (*e.g.*, hormones or neurotransmitters), proteins such as antibodies, or non-peptide molecules can be coupled to liposomes chemically.

Electroporation is another alternative mode of administration. For example, Aihara and Miyazaki, *Nature Biotechnology* 16:867 (1998), have demonstrated the use of *in vivo* electroporation for gene transfer into muscle.

In an alternative approach to gene therapy, a therapeutic gene may encode a *Zcytor21* anti-sense RNA that inhibits the expression of *Zcytor21*. Suitable sequences for anti-sense molecules can be derived from the nucleotide sequences of *Zcytor21* disclosed herein.

Alternatively, an expression vector can be constructed in which a regulatory element is operably linked to a nucleotide sequence that encodes a ribozyme. Ribozymes can be designed to express endonuclease activity that is directed to a certain target sequence in an mRNA molecule (see, for example, Draper and Macejak, U.S. Patent No. 5,496,698, McSwiggen, U.S. Patent No. 5,525,468, Chowrira and McSwiggen, U.S. Patent No. 5,631,359, and Robertson and Goldberg, U.S. Patent No. 5,225,337). In the context of the present invention, ribozymes include nucleotide sequences that bind with *Zcytor21* mRNA.

In another approach, expression vectors can be constructed in which a regulatory element directs the production of RNA transcripts capable of promoting RNase P-mediated cleavage of mRNA molecules that encode a *Zcytor21* gene. According to this approach, an external guide sequence can be constructed for directing the endogenous ribozyme, RNase P, to a particular species of intracellular mRNA, which is subsequently cleaved by the cellular ribozyme (see, for example, Altman *et al.*, U.S. Patent No. 5,160,053, Yuan *et al.*, *Science* 263:1269 (1994), Pace *et al.*, international publication No. WO 96/18733, George *et al.*, international publication No. WO 96/21731, and Werner *et al.*, international publication No. WO 97/33991). For example, the external

guide sequence can comprise a ten to fifteen nucleotide sequence complementary to *Zcytor21* mRNA, and a 3'-NCCA nucleotide sequence, wherein N is preferably a purine. The external guide sequence transcripts bind to the targeted mRNA species by the formation of base pairs between the mRNA and the complementary external guide sequences, thus promoting cleavage of mRNA by RNase P at the nucleotide located at the 5'-side of the base-paired region.

In general, the dosage of a composition comprising a therapeutic vector having a *Zcytor21* nucleotide sequence, such as a recombinant virus, will vary depending upon such factors as the subject's age, weight, height, sex, general medical condition and previous medical history. Suitable routes of administration of therapeutic vectors include intravenous injection, intraarterial injection, intraperitoneal injection, intramuscular injection, intratumoral injection, and injection into a cavity that contains a tumor. As an illustration, Horton *et al.*, *Proc. Nat'l Acad. Sci. USA* 96:1553 (1999), demonstrated that intramuscular injection of plasmid DNA encoding interferon- $\alpha$  produces potent antitumor effects on primary and metastatic tumors in a murine model.

A composition comprising viral vectors, non-viral vectors, or a combination of viral and non-viral vectors of the present invention can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby vectors or viruses are combined in a mixture with a pharmaceutically acceptable carrier. As noted above, a composition, such as phosphate-buffered saline is said to be a "pharmaceutically acceptable carrier" if its administration can be tolerated by a recipient subject. Other suitable carriers are well-known to those in the art (see, for example, *Remington's Pharmaceutical Sciences*, 19th Ed. (Mack Publishing Co. 1995), and *Gilman's the Pharmacological Basis of Therapeutics*, 7th Ed. (MacMillan Publishing Co. 1985)).

For purposes of therapy, a therapeutic gene expression vector, or a recombinant virus comprising such a vector, and a pharmaceutically acceptable carrier are administered to a subject in a therapeutically effective amount. A combination of an expression vector (or virus) and a pharmaceutically acceptable carrier is said to be administered in a "therapeutically effective amount" if the amount administered is physiologically significant. An agent is physiologically significant if its presence

results in a detectable change in the physiology of a recipient subject. For example, an agent used to treat inflammation is physiologically significant if its presence alleviates the inflammatory response.

When the subject treated with a therapeutic gene expression vector or a recombinant virus is a human, then the therapy is preferably somatic cell gene therapy. That is, the preferred treatment of a human with a therapeutic gene expression vector or a recombinant virus does not entail introducing into cells a nucleic acid molecule that can form part of a human germ line and be passed onto successive generations (*i.e.*, human germ line gene therapy).

### 13. Production of Transgenic Mice

Transgenic mice can be engineered to over-express the *Zcytor21* gene in all tissues or under the control of a tissue-specific or tissue-preferred regulatory element. These over-producers of *Zcytor21* can be used to characterize the phenotype that results from over-expression, and the transgenic animals can serve as models for human disease caused by excess *Zcytor21*. Transgenic mice that over-express *Zcytor21* also provide model bioreactors for production of *Zcytor21*, such as soluble *Zcytor21*, in the milk or blood of larger animals. Methods for producing transgenic mice are well-known to those of skill in the art (see, for example, Jacob, "Expression and Knockout of Interferons in Transgenic Mice," in *Overexpression and Knockout of Cytokines in Transgenic Mice*, Jacob (ed.), pages 111-124 (Academic Press, Ltd. 1994), Monastersky and Robl (eds.), *Strategies in Transgenic Animal Science* (ASM Press 1995), and Abbud and Nilson, "Recombinant Protein Expression in Transgenic Mice," in *Gene Expression Systems: Using Nature for the Art of Expression*, Fernandez and Hoeffler (eds.), pages 367-397 (Academic Press, Inc. 1999)).

For example, a method for producing a transgenic mouse that expresses a *Zcytor21* gene can begin with adult, fertile males (studs) (B6C3f1, 2-8 months of age (Taconic Farms, Germantown, NY)), vasectomized males (duds) (B6D2f1, 2-8 months, (Taconic Farms)), prepubescent fertile females (donors) (B6C3f1, 4-5 weeks, (Taconic Farms)) and adult fertile females (recipients) (B6D2f1, 2-4 months, (Taconic Farms)). The donors are acclimated for one week and then injected with approximately 8

IU/mouse of Pregnant Mare's Serum gonadotrophin (Sigma Chemical Company; St. Louis, MO) I.P., and 46-47 hours later, 8 IU/mouse of human Chorionic Gonadotropin (hCG (Sigma)) I.P. to induce superovulation. Donors are mated with studs subsequent to hormone injections. Ovulation generally occurs within 13 hours of hCG injection.

5 Copulation is confirmed by the presence of a vaginal plug the morning following mating.

Fertilized eggs are collected under a surgical scope. The oviducts are collected and eggs are released into urinalysis slides containing hyaluronidase (Sigma). Eggs are washed once in hyaluronidase, and twice in Whitten's W640 medium

10 (described, for example, by Menino and O'Claray, *Biol. Reprod.* 77:159 (1986), and Dienhart and Downs, *Zygote* 4:129 (1996)) that has been incubated with 5% CO<sub>2</sub>, 5% O<sub>2</sub>, and 90% N<sub>2</sub> at 37°C. The eggs are then stored in a 37°C/5% CO<sub>2</sub> incubator until microinjection.

Ten to twenty micrograms of plasmid DNA containing a *Zcytor21*

15 encoding sequence is linearized, gel-purified, and resuspended in 10 mM Tris-HCl (pH 7.4), 0.25 mM EDTA (pH 8.0), at a final concentration of 5-10 nanograms per microliter for microinjection. For example, the *Zcytor21* encoding sequences can encode a polypeptide comprising amino acid residues 24 to 454 of SEQ ID NO:11, amino acid residues 24 to 376 of SEQ ID NO:2, amino acid residues 24 to 396 of SEQ

20 ID NO:5, amino acid residues 24 to 533 of SEQ ID NO:8, or amino acid residues 24 to 444 of SEQ ID NO:15.

Plasmid DNA is microinjected into harvested eggs contained in a drop of W640 medium overlaid by warm, CO<sub>2</sub>-equilibrated mineral oil. The DNA is drawn into an injection needle (pulled from a 0.75mm ID, 1mm OD borosilicate glass

25 capillary), and injected into individual eggs. Each egg is penetrated with the injection needle, into one or both of the haploid pronuclei.

Picoliters of DNA are injected into the pronuclei, and the injection needle withdrawn without coming into contact with the nucleoli. The procedure is repeated until all the eggs are injected. Successfully microinjected eggs are transferred

30 into an organ tissue-culture dish with pre-gassed W640 medium for storage overnight in a 37°C/5% CO<sub>2</sub> incubator.

The following day, two-cell embryos are transferred into pseudopregnant recipients. The recipients are identified by the presence of copulation plugs, after copulating with vasectomized duds. Recipients are anesthetized and shaved on the dorsal left side and transferred to a surgical microscope. A small incision is made in the skin and through the muscle wall in the middle of the abdominal area outlined by the ribcage, the saddle, and the hind leg, midway between knee and spleen. The reproductive organs are exteriorized onto a small surgical drape. The fat pad is stretched out over the surgical drape, and a baby serrefine (Roboz, Rockville, MD) is attached to the fat pad and left hanging over the back of the mouse, preventing the organs from sliding back in.

With a fine transfer pipette containing mineral oil followed by alternating W640 and air bubbles, 12-17 healthy two-cell embryos from the previous day's injection are transferred into the recipient. The swollen ampulla is located and holding the oviduct between the ampulla and the bursa, a nick in the oviduct is made with a 28 g needle close to the bursa, making sure not to tear the ampulla or the bursa.

The pipette is transferred into the nick in the oviduct, and the embryos are blown in, allowing the first air bubble to escape the pipette. The fat pad is gently pushed into the peritoneum, and the reproductive organs allowed to slide in. The peritoneal wall is closed with one suture and the skin closed with a wound clip. The mice recuperate on a 37°C slide warmer for a minimum of four hours.

The recipients are returned to cages in pairs, and allowed 19-21 days gestation. After birth, 19-21 days postpartum is allowed before weaning. The weanlings are sexed and placed into separate sex cages, and a 0.5 cm biopsy (used for genotyping) is snipped off the tail with clean scissors.

Genomic DNA is prepared from the tail snips using, for example, a QIAGEN DNEASY kit following the manufacturer's instructions. Genomic DNA is analyzed by PCR using primers designed to amplify a *Zcytor21* gene or a selectable marker gene that was introduced in the same plasmid. After animals are confirmed to be transgenic, they are back-crossed into an inbred strain by placing a transgenic female with a wild-type male, or a transgenic male with one or two wild-type female(s). As

pups are born and weaned, the sexes are separated, and their tails snipped for genotyping.

To check for expression of a transgene in a live animal, a partial hepatectomy is performed. A surgical prep is made of the upper abdomen directly below the zyphoid process. Using sterile technique, a small 1.5-2 cm incision is made below the sternum and the left lateral lobe of the liver exteriorized. Using 4-0 silk, a tie is made around the lower lobe securing it outside the body cavity. An atraumatic clamp is used to hold the tie while a second loop of absorbable Dexon (American Cyanamid; Wayne, N.J.) is placed proximal to the first tie. A distal cut is made from the Dexon tie and approximately 100 mg of the excised liver tissue is placed in a sterile petri dish. The excised liver section is transferred to a 14 ml polypropylene round bottom tube and snap frozen in liquid nitrogen and then stored on dry ice. The surgical site is closed with suture and wound clips, and the animal's cage placed on a 37°C heating pad for 24 hours post operatively. The animal is checked daily post operatively and the wound clips removed 7-10 days after surgery. The expression level of *Zcytor21* mRNA is examined for each transgenic mouse using an RNA solution hybridization assay or polymerase chain reaction.

In addition to producing transgenic mice that over-express *Zcytor21*, it is useful to engineer transgenic mice with either abnormally low or no expression of the gene. Such transgenic mice provide useful models for diseases associated with a lack of *Zcytor21*. As discussed above, *Zcytor21* gene expression can be inhibited using anti-sense genes, ribozyme genes, or external guide sequence genes. To produce transgenic mice that under-express the *Zcytor21* gene, such inhibitory sequences are targeted to *Zcytor21* mRNA. Methods for producing transgenic mice that have abnormally low expression of a particular gene are known to those in the art (see, for example, Wu *et al.*, "Gene Underexpression in Cultured Cells and Animals by Antisense DNA and RNA Strategies," in *Methods in Gene Biotechnology*, pages 205-224 (CRC Press 1997)).

An alternative approach to producing transgenic mice that have little or no *Zcytor21* gene expression is to generate mice having at least one normal *Zcytor21* allele replaced by a nonfunctional *Zcytor21* gene. One method of designing a

nonfunctional *Zcytor21* gene is to insert another gene, such as a selectable marker gene, within a nucleic acid molecule that encodes *Zcytor21*. Standard methods for producing these so-called "knockout mice" are known to those skilled in the art (see, for example, Jacob, "Expression and Knockout of Interferons in Transgenic Mice," in  
5 *Overexpression and Knockout of Cytokines in Transgenic Mice*, Jacob (ed.), pages 111-124 (Academic Press, Ltd. 1994), and Wu *et al.*, "New Strategies for Gene Knockout," in *Methods in Gene Biotechnology*, pages 339-365 (CRC Press 1997)).

#### *Example 1*

##### 10 *Human Zcytor21 Tissue Distribution in Tissue Panels Using PCR*

##### *Tissue Distribution in tissue cDNA panels using PCR*

The Human Rapid-Scan cDNA panel represents 24 adult tissues and is arrayed at 4 different concentrations called 1X, 10X, 100X, and 1000X (Origen,  
15 Rockville, MD.). The "1000x and 100x" levels were screened for *zcytor21* transcription using PCR. The sense primer was *zc39334*, (5'-AGGCCCTGCCACCCACCTTC 3') (SEQ ID NO:17) located in a cDNA area corresponding to the 5' untranslated region. The antisense primer was *zc39333*, (5'-CGAGGCACCCCAAGGATTTTCAG-3') (SEQ ID NO:18) located in a cDNA area  
20 corresponding to the 3' untranslated region. PCR was applied using pfu turbo polymerase and the manufacturer's recommendations (Stratagene, La Jolla, CA) except for using rediload dye, (Research Genetics, Inc., Huntsville, AL) a wax hot start, (Molecular Bioproducts Inc. San Diego, CA) and 10% (final concentration) DMSO. The amplification was carried out as follows: 1 cycle at 94°C for 4 minutes, 40 cycles  
25 of 94°C for 30 seconds, 51°C for 30 seconds and 72°C for 3 minutes, followed by 1 cycle at 72°C for 7 minutes. About 10 µl of the PCR reaction product was subjected to standard agarose gel electrophoresis using a 1% agarose gel. Following electrophoresis, the gels were Southern blotted and the membranes hybridized by standard methods using a <sup>32</sup>P isotope-labeled oligonucleotide, *zc40458* (5'-  
30 TCTCTGACTCTGCTGGGATTGG-3') (SEQ ID NO:19) which maps to the cDNA area in the translated region, just downstream of the start codon. X ray film



autoradiography revealed zcytor21-specific amplicons only in colon, lung, stomach, placenta, and bone marrow.

5       The complete disclosure of all patents, patent applications, and  
publications, and electronically available material (e.g., GenBank amino acid and  
nucleotide sequence submissions) cited herein are incorporated by reference. The  
foregoing detailed description and examples have been given for clarity of  
understanding only. No unnecessary limitations are to be understood therefrom. The  
invention is not limited to the exact details shown and described, for variations obvious  
10   to one skilled in the art will be included within the invention defined by the claims.

## CLAIMS

What is claimed is:

1. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of amino acid residues 24 to 454 of SEQ ID NO:11, amino acid residues 24 to 376 of SEQ ID NO:2, amino acid residues 24 to 396 of SEQ ID NO:5, amino acid residues 24 to 533 of SEQ ID NO:8, and amino acid residues 24 to 444 of SEQ ID NO:15.

2. The isolated polypeptide of claim 1 wherein the isolated polypeptide comprises an amino acid sequence selected from the group consisting of amino acid residues 24 to 667 of SEQ ID NO:11, amino acid residues 24 to 589 of SEQ ID NO:2, amino acid residues 24 to 609 of SEQ ID NO:5, and amino acid residues 24 to 657 of SEQ ID NO:15.

3. The isolated polypeptide of claim 1 wherein the isolated polypeptide comprises an amino acid sequence selected from the group consisting of the amino acid sequence of SEQ ID NO:2, the amino acid sequence of SEQ ID NO:5, the amino acid sequence of SEQ ID NO:8, the amino acid sequence of SEQ ID NO:11, and the amino acid sequence of SEQ ID NO:15.

4. An isolated nucleic acid molecule encoding a polypeptide wherein the polypeptide comprises an amino acid sequence selected from the group consisting of amino acid residues 24 to 454 of SEQ ID NO:11, amino acid residues 24 to 376 of SEQ ID NO:2, amino acid residues 24 to 396 of SEQ ID NO:5, amino acid residues 24 to 533 of SEQ ID NO:8, and amino acid residues 24 to 444 of SEQ ID NO:15.

5. A vector comprising the isolated nucleic acid molecule of claim 4.

6. An expression vector comprising the isolated nucleic acid molecule of claim 4, a transcription promoter, and a transcription terminator, wherein the promoter is operably

linked with the nucleic acid molecule, and wherein the nucleic acid molecule is operably linked with the transcription terminator.

7. A recombinant host cell comprising the expression vector of claim 6 wherein the host cell is selected from the group consisting of bacterium, yeast cell, fungal cell, insect cell, avian cell, mammalian cell, and plant cell.

8. A method of producing Zcytor21 protein, the method comprising:  
culturing recombinant host cells that comprise the expression vector of claim 6, and that produce the Zcytor21 protein.

9. The method of claim 8 further comprising isolating the Zcytor21 protein from the cultured recombinant host cells.

10. An antibody or antibody fragment that specifically binds with the polypeptide of claim 1.

11. The antibody of claim 10 wherein the antibody is selected from the group consisting of a polyclonal antibody, a murine monoclonal antibody, a humanized antibody derived from a murine monoclonal antibody, and a human monoclonal antibody.

12. An anti-idiotypic antibody that specifically binds with the antibody of claim 10.

13. A fusion protein comprising the polypeptide of claim 1.

14. The fusion protein of claim 13 wherein the fusion protein further comprises an immunoglobulin moiety.

15. A composition comprising:  
an effective amount of the polypeptide of claim 1; and  
a pharmaceutically acceptable vehicle.

## SEQUENCE LISTING

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&lt;120&gt; Human Cytokine Receptor

&lt;130&gt; 01-25PC

&lt;150&gt; US 60/305,168

&lt;151&gt; 2001-07-13

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&lt;170&gt; FastSEQ for Windows Version 4.0

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 Met Gly Ser Ser Arg Leu Ala Ala Leu Leu Leu Pro Leu Leu Leu  
 1 5 10 15

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 Ile Val Ile Asp Leu Ser Asp Ser Ala Gly Ile Gly Phe Arg His Leu  
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 Pro His Trp Asn Thr Arg Cys Pro Leu Ala Ser His Thr Val Phe Asn  
 35 40 45

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 Gly Ala Ser Ser Thr Ser Trp Cys Arg Asn Pro Lys Ser Leu Pro His  
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tca agt tct ata gga gac aca aga tgc cag cac ctg ctc aga gga agc 302  
 Ser Ser Ser Ile Gly Asp Thr Arg Cys Gln His Leu Leu Arg Gly Ser  
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 Cys Cys Leu Val Val Thr Cys Leu Arg Arg Ala Ile Thr Phe Pro Ser  
 80 85 90 95

cct ccc cag aca tct ccc aca agg gac ttc gct cta aaa gga ccc aac 398  
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ctt cgg atc cag aga cat ggg aaa gtc ttc cca gat tgg act cac aaa 446  
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 115 120 125

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 Gly Pro Glu Phe Ser Phe Asp Leu Leu Pro Glu Ala Arg Ala Ile Arg  
 130 135 140

gtg acc ata tct tca ggc cct gag gtc agc gtg cgt ctt tgt cac cag	542
Val Thr Ile Ser Ser Gly Pro Glu Val Ser Val Arg Leu Cys His Gln	
145 150 155	
tgg gca ctg gag tgt gaa gag ctg agc agt ccc tat gat gtc cag aaa	590
Trp Ala Leu Glu Cys Glu Glu Leu Ser Ser Pro Tyr Asp Val Gln Lys	
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Ile Val Ser Gly Gly His Thr Val Glu Leu Pro Tyr Glu Phe Leu Leu	
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Pro Cys Leu Cys Ile Glu Ala Ser Tyr Leu Gln Glu Asp Thr Val Arg	
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Arg Lys Lys Cys Pro Phe Gln Ser Trp Pro Glu Ala Tyr Gly Ser Asp	
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Phe Trp Lys Ser Val His Phe Thr Asp Tyr Ser Gln His Thr Gln Met	
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Val Met Ala Leu Thr Arg Cys Pro Leu Lys Leu Glu Ala Ala Leu	
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Cys Gln Arg His Asp Trp His Thr Leu Cys Lys Asp Leu Pro Asn Ala	
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Thr Ala Arg Glu Ser Asp Gly Trp Tyr Val Leu Glu Lys Val Asp Leu	
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His Pro Gln Leu Cys Phe Lys Phe Ser Phe Gly Asn Ser Ser His Val	
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Glu Cys Pro His Gln Thr Gly Ile Thr Glu Ala Arg Asp Trp Pro Ser	
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His Ile Gln Val Ser Cys Ser Pro Gly Val Pro Ile Arg Glu Pro Gln	
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Thr Ser Asn Cys Leu Trp Phe Val Arg Asn Glu Ala Thr Gln Gln Glu	
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Ala Arg Gly Ser Ser Pro Val Ser Leu Asp Leu Ile Ile Pro Phe Leu	
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Leu	Ile	Leu	Ala	Leu	Leu	Ala	Leu	Leu	Thr	Leu	Leu	Gly	Val	Val	Leu		
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gcc	ctc	acc	tgc	cgg	cgc	cca	cag	tca	ggc	ccg	ggc	cca	gcg	cgg	cca	1358	
Ala	Leu	Thr	Cys	Arg	Arg	Pro	Gln	Ser	Gly	Pro	Gly	Pro	Ala	Arg	Pro		
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Val	Leu	Leu	Leu	His	Ala	Ala	Asp	Ser	Glu	Ala	Gln	Arg	Arg	Leu	Val		
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Gly	Ala	Leu	Ala	Glu	Leu	Leu	Arg	Ala	Ala	Leu	Gly	Gly	Gly	Arg	Asp		
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Val	Ile	Val	Asp	Leu	Trp	Glu	Gly	Arg	His	Val	Ala	Arg	Val	Gly	Pro		
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ctg	ccg	tgg	ctc	tgg	gcg	gcg	cgg	acg	cgc	gta	gcg	cgg	gag	cag	ggc	1550	
Leu	Pro	Trp	Leu	Trp	Ala	Ala	Arg	Thr	Arg	Val	Ala	Arg	Glu	Gln	Gly		
480					485					490					495		
act	gtg	ctg	ctg	ctg	tgg	agc	ggc	gcc	gac	ctt	cgc	ccg	gtc	agc	ggc	1598	
Thr	Val	Leu	Leu	Leu	Trp	Ser	Gly	Ala	Asp	Leu	Arg	Pro	Val	Ser	Gly		
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Pro	Asp	Pro	Arg	Ala	Ala	Pro	Leu	Leu	Ala	Leu	Leu	His	Ala	Ala	Pro		
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cgc	ccg	ctg	ctg	ctg	ctc	gct	tac	ttc	agt	cgc	ctc	tgc	gcc	aag	ggc	1694	
Arg	Pro	Leu	Leu	Leu	Leu	Ala	Tyr	Phe	Ser	Arg	Leu	Cys	Ala	Lys	Gly		
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gac	atc	ccc	ccg	ccg	ctg	cgc	gcc	ctg	ccg	cgc	tac	cgc	ctg	ctg	cgc	1742	
Asp	Ile	Pro	Pro	Pro	Leu	Arg	Ala	Leu	Pro	Arg	Tyr	Arg	Leu	Leu	Arg		
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gac	ctg	ccg	cgt	ctg	ctg	cgg	gcg	ctg	gac	gcg	cgg	cct	ttc	gca	gag	1790	
Asp	Leu	Pro	Arg	Leu	Leu	Arg	Ala	Leu	Asp	Ala	Arg	Pro	Phe	Ala	Glu		
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gcc	acc	agc	tgg	ggc	cgc	ctt	ggg	gcg	cgg	cag	cgc	agg	cag	agc	cgc	1838	
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cta	gag	ctg	tgc	agc	cgg	ctc	gaa	cga	gag	gcc	gcc	cga	ctt	gca	gac	1886	
Leu	Glu	Leu	Cys	Ser	Arg	Leu	Glu	Arg	Glu	Ala	Ala	Arg	Leu	Ala	Asp		
			595					600						605			
cta	ggg	tgagcagagc	tccaccgcag	tcccgggtgt	ctgcggccgc	aacgcaacgg										1942	
Leu	Gly																
acactggctg	gaaccccggg	atgagccttc	gaccctgaaa	tccttgggggt	gcctcg											1998	

&lt;211&gt; 609

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 5

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His Trp Asn Thr Arg Cys Pro Leu Ala Ser His Thr Val Phe Asn Gly
 35          40          45
Ala Ser Ser Thr Ser Trp Cys Arg Asn Pro Lys Ser Leu Pro His Ser
 50          55          60
Ser Ser Ile Gly Asp Thr Arg Cys Gln His Leu Leu Arg Gly Ser Cys
 65          70          75          80
Cys Leu Val Val Thr Cys Leu Arg Arg Ala Ile Thr Phe Pro Ser Pro
 85          90          95
Pro Gln Thr Ser Pro Thr Arg Asp Phe Ala Leu Lys Gly Pro Asn Leu
100          105          110
Arg Ile Gln Arg His Gly Lys Val Phe Pro Asp Trp Thr His Lys Gly
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Pro Glu Phe Ser Phe Asp Leu Leu Pro Glu Ala Arg Ala Ile Arg Val
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Thr Ile Ser Ser Gly Pro Glu Val Ser Val Arg Leu Cys His Gln Trp
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Ala Leu Glu Cys Glu Glu Leu Ser Ser Pro Tyr Asp Val Gln Lys Ile
165          170          175
Val Ser Gly Gly His Thr Val Glu Leu Pro Tyr Glu Phe Leu Leu Pro
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Cys Leu Cys Ile Glu Ala Ser Tyr Leu Gln Glu Asp Thr Val Arg Arg
195          200          205
Lys Lys Cys Pro Phe Gln Ser Trp Pro Glu Ala Tyr Gly Ser Asp Phe
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Trp Lys Ser Val His Phe Thr Asp Tyr Ser Gln His Thr Gln Met Val
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Met Ala Leu Thr Leu Arg Cys Pro Leu Lys Leu Glu Ala Ala Leu Cys
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Gln Arg His Asp Trp His Thr Leu Cys Lys Asp Leu Pro Asn Ala Thr
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Ala Arg Glu Ser Asp Gly Trp Tyr Val Leu Glu Lys Val Asp Leu His
275          280          285
Pro Gln Leu Cys Phe Lys Phe Ser Phe Gly Asn Ser Ser His Val Glu
290          295          300
Cys Pro His Gln Thr Gly Ile Thr Glu Ala Arg Asp Trp Pro Ser His
305          310          315          320
Ile Gln Val Ser Cys Ser Pro Gly Val Pro Ile Arg Glu Pro Gln Thr
325          330          335
Ser Asn Cys Leu Trp Phe Val Arg Asn Glu Ala Thr Gln Gln Glu Ala
340          345          350
Arg Gly Ser Ser Pro Val Ser Leu Asp Leu Ile Ile Pro Phe Leu Arg
355          360          365
Pro Gly Cys Cys Val Leu Val Trp Arg Ser Asp Val Gln Phe Ala Trp
370          375          380
Lys His Leu Leu Cys Pro Asp Val Ser Tyr Arg His Leu Gly Leu Leu
385          390          395          400
Ile Leu Ala Leu Leu Ala Leu Leu Thr Leu Leu Gly Val Val Leu Ala
405          410          415
Leu Thr Cys Arg Arg Pro Gln Ser Gly Pro Gly Pro Ala Arg Pro Val
420          425          430
Leu Leu Leu His Ala Ala Asp Ser Glu Ala Gln Arg Arg Leu Val Gly
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Ala Leu Ala Glu Leu Leu Arg Ala Ala Leu Gly Gly Gly Arg Asp Val  
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 Pro Trp Leu Trp Ala Ala Arg Thr Arg Val Ala Arg Glu Gln Gly Thr  
 485 490 495  
 Val Leu Leu Leu Trp Ser Gly Ala Asp Leu Arg Pro Val Ser Gly Pro  
 500 505 510  
 Asp Pro Arg Ala Ala Pro Leu Leu Ala Leu Leu His Ala Ala Pro Arg  
 515 520 525  
 Pro Leu Leu Leu Leu Ala Tyr Phe Ser Arg Leu Cys Ala Lys Gly Asp  
 530 535 540  
 Ile Pro Pro Pro Leu Arg Ala Leu Pro Arg Tyr Arg Leu Leu Arg Asp  
 545 550 555 560  
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 565 570 575  
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<211> 1827

<212> DNA

<213> Artificial Sequence

<220>

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<221> misc\_feature

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<223> n = A, T, C or G

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 gcnathmgng tnacnathws nwsnggnccn gargtnwsng tnmgnytnng ycaycartgg 480  
 gcnytngart gygargart nwsnwsnccn taygaygtnc araththgt nwsngngngn 540  
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 ytnargarg ayacngtnmg nmgnaraar tgyccnttyc arwsntggcc ngargcntay 660  
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gcnytnytn c aygcngcncc nmgnccnytn ytnytnytn g cntayttyws nmgnytntgy 1620
gcnaarggng ayathccncc nccnytnmgn gcnytnccnm gntaymgnyt nytnmngngay 1680
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<220>
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<221> misc_feature
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Met Gly Ser Ser Arg Leu Ala Ala Leu Leu Leu Pro Leu Leu Leu
1 5 10 15

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Pro His Trp Asn Thr Arg Cys Pro Leu Ala Ser His Thr Asp Asp Ser
35 40 45

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ttc cac ctc ctg gtg cag aaa tcc aaa aag tct tcc aca ttc aag ttc 398
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100 105 110

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Tyr Arg Arg His Lys Met Pro Ala Pro Ala Gln Arg Lys Leu Leu Pro
115 120 125

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cgt cgt cac ctg tct gag aag agc cat cac att tcc atc ccc tcc cca 494
Arg Arg His Leu Ser Glu Lys Ser His His Ile Ser Ile Pro Ser Pro
130 135 140

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145 150 155

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Pro Glu Thr Trp Glu Ser Leu Pro Arg Leu Asp Ser Gln Arg His Gly	
160 165 170 175	
gga ccc gag ttc tcc ttt gat ttg ctg cct gag gcc cgg gct att cgg	638
Gly Pro Glu Phe Ser Phe Asp Leu Leu Pro Glu Ala Arg Ala Ile Arg	
180 185 190	
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Val Thr Ile Ser Ser Gly Pro Glu Val Ser Val Arg Leu Cys His Gln	
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tgg gca ctg gag tgt gaa gag ctg agc agt ccc tat gat gtc cag aaa	734
Trp Ala Leu Glu Cys Glu Glu Leu Ser Ser Pro Tyr Asp Val Gln Lys	
210 215 220	
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Ile Val Ser Gly Gly His Thr Val Glu Leu Pro Tyr Glu Phe Leu Leu	
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Leu Val Pro Pro Val Tyr Thr Val Ser Gln Ala Arg Gly Ser Ser Pro  
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 Val Cys Leu Glu Ala Pro Leu Val Ser Gly Cys Leu Leu Gln Thr Pro  
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 Cys Ser Gly Pro His Leu Pro Ala Pro Thr Val Arg Pro Gly Pro Ser  
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 Pro Gly Gly Ser Ala Gly  
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 caagggcgac atccccccgc cgctgcgcgc cctgccgcgc taccgcctgc tgcgcgacct 1994  
 gccgcgtctg ctgcggggcg tggacgcgcg gcctttcgca gaggccacca gctggggccg 2054  
 ccttggggcg cggcagcgca ggcagagcgc cctagagctg tgcagccggc tcgaacgaga 2114  
 ggccgcccga cttgcagacc taggttgagc agagctccac cgcagtcgcc ggtgtctgcg 2174  
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 <212> PRT  
 <213> Homo sapiens

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 His Trp Asn Thr Arg Cys Pro Leu Ala Ser His Thr Asp Asp Ser Phe  
 35 40 45  
 Thr Gly Ser Ser Ala Tyr Ile Pro Cys Arg Thr Trp Trp Ala Leu Phe  
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 Ser Thr Lys Pro Trp Cys Val Arg Val Trp His Cys Ser Arg Cys Leu  
 65 70 75 80



[illegible]

<210>	9
<211>	1599
<212>	DNA

<213> Artificial Sequence

<220>

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<221> misc\_feature

<222> (1)...(1599)

<223> n = A, T, C or G

<400> 9

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gchwsncaya cngaygayws nttyacnggn wsnwsngcnt ayathccntg ymgnaacntgg 180
tgggcnytn tywsnacnaa rccntgggtg gtnmgngntnt ggcaytgyws nmngtgyytn 240
tgyrcarcay tnytnwsngg nggnwsnggn ytnccarmgng gnytnnttyca yytnytnngtn 300
caraarwsna araarwsnws nacnttyaar ttytaymgnm gncayaarat gccngcncn 360
gcncarmgna arytnytnc nmgnmgncay ytnwsngara arwsncayca yathwsnath 420
ccnwsnccng ayathwsnca yaarggnytn mgnwsnaarm gnacncarcc nwsngayccn 480
garacntggg arwsnytncc nmgnytnngay wsnccarmgnc ayggngggncc ngarttywsn 540
ttygayytny tncngargc nmngcncath mgngtnacna thwsnwsngg nccngargtn 600
wsngtnmgny tntgycauca rtgggcnytn gartgygarg arytnwsnws nccntaygay 660
gtncaraara thgtwnsngg nggncayacn gtngarytnc cntaygartt yytnytncn 720
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taywsncarc ayacncarat ggtnatggcn ytnacnytnm gntgyccnyt naarytnar 900
gcngcnytn gycarmgna ygaytggcay acnytnntgya argayytncc naaygcncn 960
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ttyaarttyw snttyggnaa ywsnwsncay gtngartgyc cncaycarac nggnwsnytn 1080
acnwsntgga aygtwnsnat ggayacncar gcncarcary tnathytnca yttywsnwsn 1140
mgntatgca cncanttyws ngcngcntgg wsnytncng gnytnngnca rgayacnytn 1200
gtncncncng tntayacngt nwsncargcn mgnggnwsnw snccngtnws nytngayytn 1260
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gcnytnngayc cnggnacngc nggncncn cayccnacng gntgytgyws nggncncay 1500
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<212> DNA

<213> Homo sapiens

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<222> (66)...(2066)

<221> misc\_feature

<222> (0)...(0)

<223> Zcytor21-d2

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Met Gly Ser Ser Arg Leu Ala Ala Leu Leu Leu Pro Leu Leu Leu
1 5 10 15

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Ile Val Ile Asp Leu Ser Asp Ser Ala Gly Ile Gly Phe Arg His Leu
20 25 30

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ccc cac tgg aac acc cgc tgt cct ctg gcc tcc cac acg gat gac agt	206
Pro His Trp Asn Thr Arg Cys Pro Leu Ala Ser His Thr Asp Asp Ser	
35 40 45	
ttc act gga agt tct gcc tat atc cct tgc cgc acc tgg tgg gcc ctc	254
Phe Thr Gly Ser Ser Ala Tyr Ile Pro Cys Arg Thr Trp Trp Ala Leu	
50 55 60	
ttc tcc aca aag cct tgg tgt gtg cga gtc tgg cac tgt tcc cgc tgt	302
Phe Ser Thr Lys Pro Trp Cys Val Arg Val Trp His Cys Ser Arg Cys	
65 70 75	
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Leu Cys Gln His Leu Leu Ser Gly Gly Ser Gly Leu Gln Arg Gly Leu	
80 85 90 95	
ttc cac ctc ctg gtg cag aaa tcc aaa aag tct tcc aca ttc aag ttc	398
Phe His Leu Leu Val Gln Lys Ser Lys Lys Ser Ser Thr Phe Lys Phe	
100 105 110	
tat agg aga cac aag atg cca gca cct gct cag agg aag ctg ctg cct	446
Tyr Arg Arg His Lys Met Pro Ala Pro Ala Gln Arg Lys Leu Leu Pro	
115 120 125	
cgt cgt cac ctg tct gag aag agc cat cac att tcc atc ccc tcc cca	494
Arg Arg His Leu Ser Glu Lys Ser His His Ile Ser Ile Pro Ser Pro	
130 135 140	
gac atc tcc cac aag gga ctt cgc tct aaa agg acc caa cct tcg gat	542
Asp Ile Ser His Lys Gly Leu Arg Ser Lys Arg Thr Gln Pro Ser Asp	
145 150 155	
cca gag aca tgg gaa agt ctt ccc aga ttg gac tca caa agg cat gga	590
Pro Glu Thr Trp Glu Ser Leu Pro Arg Leu Asp Ser Gln Arg His Gly	
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Gly Pro Glu Phe Ser Phe Asp Leu Leu Pro Glu Ala Arg Ala Ile Arg	
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Val Thr Ile Ser Ser Gly Pro Glu Val Ser Val Arg Leu Cys His Gln	
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Trp Ala Leu Glu Cys Glu Glu Leu Ser Ser Pro Tyr Asp Val Gln Lys	
210 215 220	
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Ile Val Ser Gly Gly His Thr Val Glu Leu Pro Tyr Glu Phe Leu Leu	
225 230 235	
ccc tgt ctg tgc ata gag gca tcc tac ctg caa gag gac act gtg agg	830
Pro Cys Leu Cys Ile Glu Ala Ser Tyr Leu Gln Glu Asp Thr Val Arg	
240 245 250 255	
cgc aaa aaa tgt ccc ttc cag agc tgg cca gaa gcc tat ggc tcg gac	878
Arg Lys Lys Cys Pro Phe Gln Ser Trp Pro Glu Ala Tyr Gly Ser Asp	
260 265 270	
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Phe	Trp	Lys	Ser	Val	His	Phe	Thr	Asp	Tyr	Ser	Gln	His	Thr	Gln	Met	
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Val	Met	Ala	Leu	Thr	Leu	Arg	Cys	Pro	Leu	Lys	Leu	Glu	Ala	Ala	Leu	
		290					295					300				
tgc	cag	agg	cac	gac	tgg	cat	acc	ctt	tgc	aaa	gac	ctc	ccg	aat	gcc	1022
Cys	Gln	Arg	His	Asp	Trp	His	Thr	Leu	Cys	Lys	Asp	Leu	Pro	Asn	Ala	
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acg	gct	cga	gag	tca	gat	ggg	tgg	tat	gtt	ttg	gag	aag	gtg	gac	ctg	1070
Thr	Ala	Arg	Glu	Ser	Asp	Gly	Trp	Tyr	Val	Leu	Glu	Lys	Val	Asp	Leu	
320					325					330					335	
cac	ccc	cag	ctc	tgc	ttc	aag	ttc	tct	ttt	gga	aac	agc	agc	cat	gtt	1118
His	Pro	Gln	Leu	Cys	Phe	Lys	Phe	Ser	Phe	Gly	Asn	Ser	Ser	His	Val	
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gaa	tgc	ccc	cac	cag	act	ggg	tct	ctc	aca	tcc	tgg	aat	gta	agc	atg	1166
Glu	Cys	Pro	His	Gln	Thr	Gly	Ser	Leu	Thr	Ser	Trp	Asn	Val	Ser	Met	
			355					360					365			
gat	acc	caa	gcc	cag	cag	ctg	att	ctt	cac	ttc	tcc	tca	aga	atg	cat	1214
Asp	Thr	Gln	Ala	Gln	Gln	Leu	Ile	Leu	His	Phe	Ser	Ser	Arg	Met	His	
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gcc	acc	ttc	agt	gct	gcc	tgg	agc	ctc	cca	ggc	ttg	ggg	cag	gac	act	1262
Ala	Thr	Phe	Ser	Ala	Ala	Trp	Ser	Leu	Pro	Gly	Leu	Gly	Gln	Asp	Thr	
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Leu	Val	Pro	Pro	Val	Tyr	Thr	Val	Ser	Gln	Ala	Arg	Gly	Ser	Ser	Pro	
400					405					410					415	
gtg	tca	cta	gac	ctc	atc	att	ccc	ttc	ctg	agg	cca	ggg	tgc	tgt	gtc	1358
Val	Ser	Leu	Asp	Leu	Ile	Ile	Pro	Phe	Leu	Arg	Pro	Gly	Cys	Cys	Val	
				420					425					430		
ctg	gtg	tgg	cgg	tca	gat	gtc	cag	ttt	gcc	tgg	aag	cac	ctc	ttg	tgt	1406
Leu	Val	Trp	Arg	Ser	Asp	Val	Gln	Phe	Ala	Trp	Lys	His	Leu	Leu	Cys	
			435				440						445			
cca	gat	gtc	tct	tac	aga	cac	ctg	ggg	ctc	ttg	atc	ctg	gca	ctg	ctg	1454
Pro	Asp	Val	Ser	Tyr	Arg	His	Leu	Gly	Leu	Leu	Ile	Leu	Ala	Leu	Leu	
		450					455					460				
gcc	ctc	ctc	acc	cta	ctg	ggt	gtt	gtt	ctg	gcc	ctc	acc	tgc	cgg	cgc	1502
Ala	Leu	Leu	Thr	Leu	Leu	Gly	Val	Val	Leu	Ala	Leu	Thr	Cys	Arg	Arg	
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Pro	Gln	Ser	Gly	Pro	Gly	Pro	Ala	Arg	Pro	Val	Leu	Leu	Leu	His	Ala	
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gcg	gac	tcg	gag	gcg	cag	cgg	cgc	ctg	gtg	gga	gcg	ctg	gct	gaa	ctg	1598
Ala	Asp	Ser	Glu	Ala	Gln	Arg	Arg	Leu	Val	Gly	Ala	Leu	Ala	Glu	Leu	
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cta	cgg	gca	gcg	ctg	ggc	ggc	ggg	cgc	gac	gtg	atc	gtg	gac	ctg	tgg	1646

Leu Arg Ala Ala Leu Gly Gly Gly Arg Asp Val Ile Val Asp Leu Trp  
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 Glu Gly Arg His Val Ala Arg Val Gly Pro Leu Pro Trp Leu Trp Ala  
 530 535 540  
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 Ala Arg Thr Arg Val Ala Arg Glu Gln Gly Thr Val Leu Leu Leu Trp  
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 Pro Leu Leu Ala Leu Leu His Ala Ala Pro Arg Pro Leu Leu Leu Leu  
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 Ala Tyr Phe Ser Arg Leu Cys Ala Lys Gly Asp Ile Pro Pro Pro Leu  
 595 600 605  
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 Arg Ala Leu Pro Arg Tyr Arg Leu Leu Arg Asp Leu Pro Arg Leu Leu  
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 625 630 635  
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 660 665  
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 His Trp Asn Thr Arg Cys Pro Leu Ala Ser His Thr Asp Asp Ser Phe  
 35 40 45  
 Thr Gly Ser Ser Ala Tyr Ile Pro Cys Arg Thr Trp Trp Ala Leu Phe  
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 Ser Thr Lys Pro Trp Cys Val Arg Val Trp His Cys Ser Arg Cys Leu  
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 Cys Gln His Leu Leu Ser Gly Gly Ser Gly Leu Gln Arg Gly Leu Phe  
 85 90 95

His Leu Leu Val Gln Lys Ser Lys Lys Ser Ser Thr Phe Lys Phe Tyr  
 100 105 110  
 Arg Arg His Lys Met Pro Ala Pro Ala Gln Arg Lys Leu Leu Pro Arg  
 115 120 125  
 Arg His Leu Ser Glu Lys Ser His His Ile Ser Ile Pro Ser Pro Asp  
 130 135 140  
 Ile Ser His Lys Gly Leu Arg Ser Lys Arg Thr Gln Pro Ser Asp Pro  
 145 150 155 160  
 Glu Thr Trp Glu Ser Leu Pro Arg Leu Asp Ser Gln Arg His Gly Gly  
 165 170 175  
 Pro Glu Phe Ser Phe Asp Leu Leu Pro Glu Ala Arg Ala Ile Arg Val  
 180 185 190  
 Thr Ile Ser Ser Gly Pro Glu Val Ser Val Arg Leu Cys His Gln Trp  
 195 200 205  
 Ala Leu Glu Cys Glu Glu Leu Ser Ser Pro Tyr Asp Val Gln Lys Ile  
 210 215 220  
 Val Ser Gly Gly His Thr Val Glu Leu Pro Tyr Glu Phe Leu Leu Pro  
 225 230 235 240  
 Cys Leu Cys Ile Glu Ala Ser Tyr Leu Gln Glu Asp Thr Val Arg Arg  
 245 250 255  
 Lys Lys Cys Pro Phe Gln Ser Trp Pro Glu Ala Tyr Gly Ser Asp Phe  
 260 265 270  
 Trp Lys Ser Val His Phe Thr Asp Tyr Ser Gln His Thr Gln Met Val  
 275 280 285  
 Met Ala Leu Thr Leu Arg Cys Pro Leu Lys Leu Glu Ala Ala Leu Cys  
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 Gln Arg His Asp Trp His Thr Leu Cys Lys Asp Leu Pro Asn Ala Thr  
 305 310 315 320  
 Ala Arg Glu Ser Asp Gly Trp Tyr Val Leu Glu Lys Val Asp Leu His  
 325 330 335  
 Pro Gln Leu Cys Phe Lys Phe Ser Phe Gly Asn Ser Ser His Val Glu  
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 Cys Pro His Gln Thr Gly Ser Leu Thr Ser Trp Asn Val Ser Met Asp  
 355 360 365  
 Thr Gln Ala Gln Gln Leu Ile Leu His Phe Ser Ser Arg Met His Ala  
 370 375 380  
 Thr Phe Ser Ala Ala Trp Ser Leu Pro Gly Leu Gly Gln Asp Thr Leu  
 385 390 395 400  
 Val Pro Pro Val Tyr Thr Val Ser Gln Ala Arg Gly Ser Ser Pro Val  
 405 410 415  
 Ser Leu Asp Leu Ile Ile Pro Phe Leu Arg Pro Gly Cys Cys Val Leu  
 420 425 430  
 Val Trp Arg Ser Asp Val Gln Phe Ala Trp Lys His Leu Leu Cys Pro  
 435 440 445  
 Asp Val Ser Tyr Arg His Leu Gly Leu Leu Ile Leu Ala Leu Leu Ala  
 450 455 460  
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 465 470 475 480  
 Gln Ser Gly Pro Gly Pro Ala Arg Pro Val Leu Leu Leu His Ala Ala  
 485 490 495  
 Asp Ser Glu Ala Gln Arg Arg Leu Val Gly Ala Leu Ala Glu Leu Leu  
 500 505 510  
 Arg Ala Ala Leu Gly Gly Gly Arg Asp Val Ile Val Asp Leu Trp Glu  
 515 520 525  
 Gly Arg His Val Ala Arg Val Gly Pro Leu Pro Trp Leu Trp Ala Ala  
 530 535 540  
 Arg Thr Arg Val Ala Arg Glu Gln Gly Thr Val Leu Leu Leu Trp Ser  
 545 550 555 560  
 Gly Ala Asp Leu Arg Pro Val Ser Gly Pro Asp Pro Arg Ala Ala Pro  
 565 570 575

Leu Leu Ala Leu Leu His Ala Ala Pro Arg Pro Leu Leu Leu Leu Ala  
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 Tyr Phe Ser Arg Leu Cys Ala Lys Gly Asp Ile Pro Pro Pro Leu Arg  
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 Ala Leu Pro Arg Tyr Arg Leu Leu Arg Asp Leu Pro Arg Leu Leu Arg  
                   610                                  615                                  620  
 Ala Leu Asp Ala Arg Pro Phe Ala Glu Ala Thr Ser Trp Gly Arg Leu  
                   625                                  630                                  635                                  640  
 Gly Ala Arg Gln Arg Arg Gln Ser Arg Leu Glu Leu Cys Ser Arg Leu  
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<210> 12

<211> 2001

<212> DNA

<213> Artificial Sequence

<220>

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<221> misc\_feature

<222> (1)...(2001)

<223> n = A, T, C or G

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2001

<210> 13  
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 <212> PRT  
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<220>  
 <223> Peptide linker

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<210> 14  
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 <212> DNA  
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<221> misc\_feature  
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 1 5 10 15

gtc atc gac ctc tct gac tct gct ggg att ggc ttt cgc cac ctg ccc 96  
 Val Ile Asp Leu Ser Asp Ser Ala Gly Ile Gly Phe Arg His Leu Pro  
 20 25 30

cac tgg aac acc cgc tgt cct ctg gcc tcc cac acg gaa gtt ctg cct 144  
 His Trp Asn Thr Arg Cys Pro Leu Ala Ser His Thr Glu Val Leu Pro  
 35 40 45

ata tcc ctt gcc gca cct ggt ggg ccc tct tct cca caa agc ctt ggt 192  
 Ile Ser Leu Ala Ala Pro Gly Gly Pro Ser Ser Pro Gln Ser Leu Gly  
 50 55 60

gtg tgc gag tct ggc act gtt ccc gct gtt tgt gcc agc atc tgc tgt 240  
 Val Cys Glu Ser Gly Thr Val Pro Ala Val Cys Ala Ser Ile Cys Cys  
 65 70 75 80

cag gtg gct cag gtc ttc aac ggg gcc tct tcc acc tcc tgg tgc aga 288  
 Gln Val Ala Gln Val Phe Asn Gly Ala Ser Ser Thr Ser Trp Cys Arg  
 85 90 95

aat cca aaa agt ctt cca cat tca agt tct ata gga gac aca aga tgc 336  
 Asn Pro Lys Ser Leu Pro His Ser Ser Ser Ile Gly Asp Thr Arg Cys  
 100 105 110

cag cac ctg ctc aga gga agc tgc tgc ctc gtc gtc acc tgt ctg aga 384  
 Gln His Leu Leu Arg Gly Ser Cys Cys Leu Val Val Thr Cys Leu Arg  
 115 120 125

aga gcc atc aca ttt cca tcc cct ccc cag aca tct ccc aca agg gac 432  
 Arg Ala Ile Thr Phe Pro Ser Pro Pro Gln Thr Ser Pro Thr Arg Asp  
 130 135 140



ttc gct cta aaa gga ccc aac ctt cgg atc cag aga cat ggg aaa gtc	480
Phe Ala Leu Lys Gly Pro Asn Leu Arg Ile Gln Arg His Gly Lys Val	
145 150 155 160	
ttc cca gat tgg act cac aaa ggc atg gag gtg ggc act ggg tac aac	528
Phe Pro Asp Trp Thr His Lys Gly Met Glu Val Gly Thr Gly Tyr Asn	
165 170 175	
agg aga tgg gtt cag ctg agt ggt gga ccc gag ttc tcc ttt gat ttg	576
Arg Arg Trp Val Gln Leu Ser Gly Gly Pro Glu Phe Ser Phe Asp Leu	
180 185 190	
ctg cct gag gcc cgg gct att cgg gtg acc ata tct tca ggc cct gag	624
Leu Pro Glu Ala Arg Ala Ile Arg Val Thr Ile Ser Ser Gly Pro Glu	
195 200 205	
gtc agc gtg cgt ctt tgt cac cag tgg gca ctg gag tgt gaa gag ctg	672
Val Ser Val Arg Leu Cys His Gln Trp Ala Leu Glu Cys Glu Glu Leu	
210 215 220	
agc agt ccc tat gat gtc cag aaa att gtg tct ggg ggc cac act gta	720
Ser Ser Pro Tyr Asp Val Gln Lys Ile Val Ser Gly Gly His Thr Val	
225 230 235 240	
gag ctg cct tat gaa ttc ctt ctg ccc tgt ctg tgc ata gag gca tcc	768
Glu Leu Pro Tyr Glu Phe Leu Leu Pro Cys Leu Cys Ile Glu Ala Ser	
245 250 255	
tac ctg caa gag gac act gtg agg cgc aaa aaa tgt ccc ttc cag agc	816
Tyr Leu Gln Glu Asp Thr Val Arg Arg Lys Lys Cys Pro Phe Gln Ser	
260 265 270	
tgg cca gaa gcc tat ggc tcg gac ttc tgg aag tca gtg cac ttc act	864
Trp Pro Glu Ala Tyr Gly Ser Asp Phe Trp Lys Ser Val His Phe Thr	
275 280 285	
gac tac agc cag cac act cag atg gtc atg gcc ctg aca ctc cgc tgc	912
Asp Tyr Ser Gln His Thr Gln Met Val Met Ala Leu Thr Leu Arg Cys	
290 295 300	
cca ctg aag ctg gaa gct gcc ctc tgc cag agg cac gac tgg cat acc	960
Pro Leu Lys Leu Glu Ala Ala Leu Cys Gln Arg His Asp Trp His Thr	
305 310 315 320	
ctt tgc aaa gac ctc ccg aat gcc acg gct cga gag tca gat ggg tgg	1008
Leu Cys Lys Asp Leu Pro Asn Ala Thr Ala Arg Glu Ser Asp Gly Trp	
325 330 335	
tat gtt ttg gag aag gtg gac ctg cac ccc cag ctc tgc ttc aag gta	1056
Tyr Val Leu Glu Lys Val Asp Leu His Pro Gln Leu Cys Phe Lys Val	
340 345 350	
caa cca tgg ttc tct ttt gga aac agc agc cat gtt gaa tgc ccc cac	1104
Gln Pro Trp Phe Ser Phe Gly Asn Ser Ser His Val Glu Cys Pro His	
355 360 365	
cag act ggg tct ctc aca tcc tgg aat gta agc atg gat acc caa gcc	1152
Gln Thr Gly Ser Leu Thr Ser Trp Asn Val Ser Met Asp Thr Gln Ala	
370 375 380	
cag cag ctg att ctt cac ttc tcc tca aga atg cat gcc acc ttc agt	1200

Gln Gln Leu Ile Leu His Phe Ser Ser Arg Met His Ala Thr Phe Ser	385	390	395	400	
gct gcc tgg agc ctc cca ggc ttg ggg cag gac act ttg gtg ccc ccc	1248				
Ala Ala Trp Ser Leu Pro Gly Leu Gly Gln Asp Thr Leu Val Pro Pro		405	410	415	
gtg tac act gtc agc cag gtg ttg cgg tca gat gtc cag ttt gcc ttg	1296				
Val Tyr Thr Val Ser Gln Val Trp Arg Ser Asp Val Gln Phe Ala Trp		420	425	430	
aag cac ctc ttg tgt cca gat gtc tct tac aga cac ctg ggg ctc ttg	1344				
Lys His Leu Leu Cys Pro Asp Val Ser Tyr Arg His Leu Gly Leu Leu		435	440	445	
atc ctg gca ctg ctg gcc ctc ctc acc cta ctg ggt gtt gtt ctg gcc	1392				
Ile Leu Ala Leu Leu Ala Leu Leu Thr Leu Leu Gly Val Val Leu Ala		450	455	460	
ctc acc tgc cgg cgc cca cag tca ggc ccg ggc cca gcg cgg cca gtg	1440				
Leu Thr Cys Arg Arg Pro Gln Ser Gly Pro Gly Pro Ala Arg Pro Val		465	470	475	480
ctc ctc ctg cac gcg gcg gac tcg gag gcg cag cgg cgc ctg gtg gga	1488				
Leu Leu Leu His Ala Ala Asp Ser Glu Ala Gln Arg Arg Leu Val Gly		485	490	495	
gcg ctg gct gaa ctg cta cgg gca gcg ctg ggc ggc ggg cgc gac gtg	1536				
Ala Leu Ala Glu Leu Leu Arg Ala Ala Leu Gly Gly Gly Arg Asp Val		500	505	510	
atc gtg gac ctg tgg gag ggg agg cac gtg gcg cgc gtg ggc ccg ctg	1584				
Ile Val Asp Leu Trp Glu Gly Arg His Val Ala Arg Val Gly Pro Leu		515	520	525	
ccg tgg ctc tgg gcg gcg cgg acg cgc gta gcg cgg gag cag ggc act	1632				
Pro Trp Leu Trp Ala Ala Thr Arg Val Ala Arg Glu Gln Gly Thr		530	535	540	
gtg ctg ctg ctg tgg agc ggc gcc gac ctt cgc ccg gtc agc ggc ccc	1680				
Val Leu Leu Leu Trp Ser Gly Ala Asp Leu Arg Pro Val Ser Gly Pro		545	550	555	560
gac ccc cgc gcc gcg ccc ctg ctc gcc ctg ctc cac gct gcc ccg cgc	1728				
Asp Pro Arg Ala Ala Pro Leu Leu Ala Leu Leu His Ala Ala Pro Arg		565	570	575	
ccg ctg ctg ctg ctc gct tac ttc agt cgc ctc tgc gcc aag ggc gac	1776				
Pro Leu Leu Leu Leu Ala Tyr Phe Ser Arg Leu Cys Ala Lys Gly Asp		580	585	590	
atc ccc ccg ccg ctg cgc gcc ctg ccg cgc tac cgc ctg ctg cgc gac	1824				
Ile Pro Pro Pro Leu Arg Ala Leu Pro Arg Tyr Arg Leu Leu Arg Asp		595	600	605	
ctg ccg cgt ctg ctg cgg gcg ctg gac gcg cgg cct ttc gca gag gcc	1872				
Leu Pro Arg Leu Leu Arg Ala Leu Asp Ala Arg Pro Phe Ala Glu Ala		610	615	620	
acc agc tgg ggc cgc ctt ggg gcg cgg cag cgc agg cag agc cgc cta	1920				
Thr Ser Trp Gly Arg Leu Gly Ala Arg Gln Arg Arg Gln Ser Arg Leu		625	630	635	640

gag ctg tgc agc cgg ctt gaa cga gag gcc gcc cga ctt gca gac cta 1968  
 Glu Leu Cys Ser Arg Leu Glu Arg Glu Ala Ala Arg Leu Ala Asp Leu  
                     645                      650                      655

ggt tga 1974  
 Gly

<210> 15  
 <211> 657  
 <212> PRT  
 <213> Homo sapiens

<400> 15  
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 Val Ile Asp Leu Ser Asp Ser Ala Gly Ile Gly Phe Arg His Leu Pro  
                     20                    25                    30  
 His Trp Asn Thr Arg Cys Pro Leu Ala Ser His Thr Glu Val Leu Pro  
                     35                    40                    45  
 Ile Ser Leu Ala Ala Pro Gly Gly Pro Ser Ser Pro Gln Ser Leu Gly  
   50                    55                    60  
 Val Cys Glu Ser Gly Thr Val Pro Ala Val Cys Ala Ser Ile Cys Cys  
   65                    70                    75                    80  
 Gln Val Ala Gln Val Phe Asn Gly Ala Ser Ser Thr Ser Trp Cys Arg  
                     85                    90                    95  
 Asn Pro Lys Ser Leu Pro His Ser Ser Ser Ile Gly Asp Thr Arg Cys  
                     100                    105                    110  
 Gln His Leu Leu Arg Gly Ser Cys Cys Leu Val Val Thr Cys Leu Arg  
                     115                    120                    125  
 Arg Ala Ile Thr Phe Pro Ser Pro Pro Gln Thr Ser Pro Thr Arg Asp  
   130                    135                    140  
 Phe Ala Leu Lys Gly Pro Asn Leu Arg Ile Gln Arg His Gly Lys Val  
   145                    150                    155                    160  
 Phe Pro Asp Trp Thr His Lys Gly Met Glu Val Gly Thr Gly Tyr Asn  
                     165                    170                    175  
 Arg Arg Trp Val Gln Leu Ser Gly Gly Pro Glu Phe Ser Phe Asp Leu  
                     180                    185                    190  
 Leu Pro Glu Ala Arg Ala Ile Arg Val Thr Ile Ser Ser Gly Pro Glu  
                     195                    200                    205  
 Val Ser Val Arg Leu Cys His Gln Trp Ala Leu Glu Cys Glu Glu Leu  
   210                    215                    220  
 Ser Ser Pro Tyr Asp Val Gln Lys Ile Val Ser Gly Gly His Thr Val  
   225                    230                    235                    240  
 Glu Leu Pro Tyr Glu Phe Leu Leu Pro Cys Leu Cys Ile Glu Ala Ser  
                     245                    250                    255  
 Tyr Leu Gln Glu Asp Thr Val Arg Arg Lys Lys Cys Pro Phe Gln Ser  
                     260                    265                    270  
 Trp Pro Glu Ala Tyr Gly Ser Asp Phe Trp Lys Ser Val His Phe Thr  
                     275                    280                    285  
 Asp Tyr Ser Gln His Thr Gln Met Val Met Ala Leu Thr Leu Arg Cys  
   290                    295                    300  
 Pro Leu Lys Leu Glu Ala Ala Leu Cys Gln Arg His Asp Trp His Thr  
   305                    310                    315                    320  
 Leu Cys Lys Asp Leu Pro Asn Ala Thr Ala Arg Glu Ser Asp Gly Trp  
                     325                    330                    335  
 Tyr Val Leu Glu Lys Val Asp Leu His Pro Gln Leu Cys Phe Lys Val  
                     340                    345                    350  
 Gln Pro Trp Phe Ser Phe Gly Asn Ser Ser His Val Glu Cys Pro His  
                     355                    360                    365  
 Gln Thr Gly Ser Leu Thr Ser Trp Asn Val Ser Met Asp Thr Gln Ala  
   370                    375                    380

Gln Gln Leu Ile Leu His Phe Ser Ser Arg Met His Ala Thr Phe Ser  
 385 390 395 400  
 Ala Ala Trp Ser Leu Pro Gly Leu Gly Gln Asp Thr Leu Val Pro Pro  
 405 410 415  
 Val Tyr Thr Val Ser<sub>2</sub> Gln Val Trp Arg Ser Asp Val Gln Phe Ala Trp  
 420 425 430  
 Lys His Leu Leu Cys Pro Asp Val Ser Tyr Arg His Leu Gly Leu Leu  
 435 440 445  
 Ile Leu Ala Leu Leu Ala Leu Leu Thr Leu Leu Gly Val Val Leu Ala  
 450 455 460  
 Leu Thr Cys Arg Arg Pro Gln Ser Gly Pro Gly Pro Ala Arg Pro Val  
 465 470 475 480  
 Leu Leu Leu His Ala Ala Asp Ser Glu Ala Gln Arg Arg Leu Val Gly  
 485 490 495  
 Ala Leu Ala Glu Leu Leu Arg Ala Ala Leu Gly Gly Gly Arg Asp Val  
 500 505 510  
 Ile Val Asp Leu Trp Glu Gly Arg His Val Ala Arg Val Gly Pro Leu  
 515 520 525  
 Pro Trp Leu Trp Ala Ala Arg Thr Arg Val Ala Arg Glu Gln Gly Thr  
 530 535 540  
 Val Leu Leu Leu Trp Ser Gly Ala Asp Leu Arg Pro Val Ser Gly Pro  
 545 550 555 560  
 Asp Pro Arg Ala Ala Pro Leu Leu Ala Leu His Ala Ala Pro Arg  
 565 570 575  
 Pro Leu Leu Leu Leu Ala Tyr Phe Ser Arg Leu Cys Ala Lys Gly Asp  
 580 585 590  
 Ile Pro Pro Pro Leu Arg Ala Leu Pro Arg Tyr Arg Leu Leu Arg Asp  
 595 600 605  
 Leu Pro Arg Leu Leu Arg Ala Leu Asp Ala Arg Pro Phe Ala Glu Ala  
 610 615 620  
 Thr Ser Trp Gly Arg Leu Gly Ala Arg Gln Arg Arg Gln Ser Arg Leu  
 625 630 635 640  
 Glu Leu Cys Ser Arg Leu Glu Arg Glu Ala Ala Arg Leu Ala Asp Leu  
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 Gly

&lt;210&gt; 16

&lt;211&gt; 1971

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Degenerate nucleotide sequence encoding the amino  
acid sequence of SEQ ID NO:15.

&lt;221&gt; misc\_feature

&lt;222&gt; (1)...(1971)

&lt;223&gt; n = A, T, C or G

&lt;400&gt; 16

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 wsnaywsng cnggnathgg nttymgncay ytnccncayt ggaayacnmg ntgyccnytn 120  
 gcnwsncaya cngargtnyt nccnathwsn ytngcngcnc cnggnggncc nwsnwsnccn 180  
 carwsnytn gngtntgyga rwsnggnacn gtnccngcng tntgygcnws nathtgytgy 240  
 cargingcnc argtnttyaa yggngcnwsn wsnacnwsnt ggtgymgnaa yccnaarwsn 300  
 ytnccncayw snwsnwsnat hggngayacn mgntgyarc ayytnytnmg nggnwsntgy 360  
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 ccnacnmng aytytgcnyt naarggnccn ayytnmgna thcarmgna yggnaargtn 480  
 ttyccngayt ggacncayaa rggnatggar gtnggnaacng gntayaaymg nmgnatgggtg 540  
 carytnwsng gnggncnga rttysntty gayytnytn cngargcnmg ngcnathmgn 600

gtnacnathw snwsnggncc ngargtnwsn gtnmgnytn gycaycartg ggcnytn gar 660  
 tgygargary tnwsnwsncc ntaygaygn caraarathg tnwsnggnng ncayacngtn 720  
 garytnccnt aygarttyyt nytnccntgy ytntgyathg argcnwsnta yytnccargar 780  
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 ttytggaarw sngtncaytt yacngaytay wsncarcaya cncaratggt natggcnyn 900  
 acnytnmgnt gycnytnaa rytngargcn gcnytnngyc armgnca yga ytggcayacn 960  
 ytntgyaarg ayytnccnaa ygcnacngcn mgngarwsng ayggntggta ygtynytn gar 1020  
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 wsnwsncayg tngartgycc ncaycaracn ggnwsnytna cnwsntggaa ygtnwsnatg 1140  
 gayacncarg cncarcaryt nathytnca y ttwysnwsnm gnatgcaygc nacnttywsn 1200  
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 ccnmngntaym gnytnytnmg ngaytnccn mgnytnytn gngcnyn g ycnmgncn 1860  
 ttygcngarg cnacnwsntg gggngmnytn ggngcnmgnc armgnmgnc rwsnmngnytn 1920  
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&lt;210&gt; 17

&lt;211&gt; 20

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; zc39334

&lt;400&gt; 17

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20

&lt;210&gt; 18

&lt;211&gt; 22

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; zc39333

&lt;400&gt; 18

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22

&lt;210&gt; 19

&lt;211&gt; 22

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; zc40458

&lt;400&gt; 19

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22